UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

O TEMPERAMENTO DE BOVINOS CONFINADOS EM DIFERENTES DISPONIBILIDADES DE ESPAÇO E SUAS RELAÇÕES COM O DESEMPENHO E A QUALIDADE DA CARCAÇA E DA CARNE

Janaina da Silva Braga

Médica Veterinária

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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 Doutor em Zootecnia.

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DADOS CURRICULARES DA AUTORA

JANAINA DA SILVA BRAGA – Nascida em 14 de novembro de 1986, na cidade de Recife – PE. Formada em Medicina Veterinária pela Universidade Federal de Uberlândia (UFU) no ano de 2009. Durante a graduação foi bolsista de iniciação científica FAPEMIG e CNPq. Iniciou seus estudos em etologia e bem-estar animal em 2006, pesquisando a interação homem-animal. A partir de 2008 passou a fazer parte do Laboratório de Bem-estar Animal (LABEA) da Universidade Federal do Paraná (UFPR), coordenado pela Profa. Dra. Carla Forte Maiolino Molento, onde concluiu seu mestrado no Programa de Pós-Graduação em Ciências Veterinárias na mesma universidade, no ano de 2010. Em 2011, se tornou integrante do Grupo de Estudos e Pesquisas em Etologia e Ecologia Animal (ETCO) na FCAV - Unesp, câmpus de Jaboticabal, onde foi bolsista da categoria Desenvolvimento Tecnológico e Industrial (DTI-B) do CNPq no projeto intitulado “Avaliação do bem-estar de bovinos de corte e definição de protocolos de boas práticas de manejo”. Em 2012 iniciou o curso de Doutorado no Programa de Pós-Graduação em Zootecnia, da FCAV - Unesp. Atualmente atua nas áreas de etologia aplicada e bem-estar de bovinos de corte, com foco nas relações entre temperamento e capacidade adaptativa aos sistemas intensivos de produção.

“If an animal population is faced with a new environment, certain selective pressures will be placed on that population and, over evolutionary time, successful adaptation may occur. If the population survives and thrives then, by definition, it has adapted. In the particular environment to which they are adapted, animals probably do not suffer. Can the same be said for farm animals? Has artificial selection (and natural selection operating in captivity) created livestock uniquely adapted to confinement? Firstly, what is adaptation?” *Tennessen, T., 1989. In: “Coping with confinement features of the environment that influence animals' ability to adapt (original paper published on Applied Animal Behaviour Science, v. 22, p. 139 – 149, 1989).*

Um homem e uma mulher, nordestinos de sotaque arrastado, de gosto simples, de braços fortes e fonte inesgotável de amor!

Aos meus pais, Marcos e Edna, eu dedico.

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Aos animais, eu ofereço.

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RESUMO – O objetivo foi avaliar os efeitos do temperamento de bovinos confinados em diferentes disponibilidades de espaço (6, 12, e 24 m^2/animal) no desempenho, na qualidade da carcaça e da carne bem como no bem-estar animal. Como objetivos específicos, destacamos: 1) avaliar os efeitos do espaço disponível no temperamento; 2) avaliar os efeitos do temperamento no desempenho e na qualidade da carcaça e da carne de bovinos confinados em diferentes disponibilidades de espaço; 3) avaliar os efeitos do temperamento na morfometria das glândulas adrenais e suas relações com a disponibilidade de espaço; 4) determinar a relação entre a morfometria das glândulas adrenais e a severidade dos hematomas nas carcaças bovinas, em diferentes disponibilidades de espaço. Foram avaliados 1.350 machos inteiros (450 Nelore e 900 cruzados Angus ou Caracu x Nelore) criados no pasto e terminados em um confinamento comercial. Temperamento foi avaliado pelo teste de velocidade de saída (VS) nos dias 0 (VS_0), 35 (VS_{35}) e 87 (ou dia final: VS_{fin}) do período de confinamento. Desempenho, características de qualidade da carcaça, pH_{24h} da carne, morfometria da adrenal e severidade dos hematomas nas carcaças foram avaliados. A VS diminuiu ao longo do período de confinamento ($p < 0.01$). No geral, VS não diferiu entre os animais mantidos em diferentes disponibilidades de espaço ($p = 0.79$) nem entre raças ($p = 0.16$). Interação entre espaço disponível e raça ou entre espaço disponível e VS_0 ou VS_{fin} não apresentaram nenhum efeito sobre as variáveis de desempenho ou características de qualidade de carcaça e carne ($p > 0.05$). Entretanto, animais calmos de acordo com a VS_{fin} tiveram maior ganho de peso do dia 0 ao 35 ($p < 0.0001$) e estavam mais pesados no dia 35º ($p < 0.0001$) e 87º ($p < 0.01$) dia do confinamento e, consequentemente, produziram carcaças mais pesadas ($p < 0.01$) comparados aos intermediários e reativos. Independente do espaço disponível e da raça, animais reativos de acordo com VS_0 apresentaram glândulas adrenais mais pesadas ($p < 0.01$) e com maior perímetro ($p = 0.01$) quando comparados com os calmos. Adicionalmente quando mantidos em reduzidos espaços disponíveis (6 ou 12 m^2 por animal), os animais reativos apresentaram maior área cortical. Com relação à severidade dos hematomas, animais com glândulas adrenais com maior área medular, maior perímetro e mais pesadas ($p < 0.05$), mantidos em 6 ou 24 m^2 por animal apresentaram maior severidade dos hematomas nas carcaças. Concluímos que as condições de confinamento aplicadas nesse estudo melhoraram o temperamento dos bovinos ao longo do tempo e que, independente do espaço disponível e da raça, os animais calmos apresentaram melhor desempenho produtivo do que os intermediários e reativos. A capacidade do animal em adaptar-se ao sistema de produção intensiva contribuiu favoravelmente para a qualidade da produção. Ainda, reduzido espaço disponível apresentou mais efeitos deletérios no bem-estar de animais reativos do que dos calmos. Além disso, animais mais estressados são mais prováveis de apresentar hematomas severos nas carcaças.

Palavras-chave: ambiente, confinamento, velocidade de saída, reatividade, Zebuínos.

TEMPERAMENT OF CATTLE CONFINED IN DIFFERENCE SPACE ALLOWANCES AND ITS RELATIONSHIPS WITH GROWTH PERFORMANCE, CARCASS AND MEAT QUALITY TRAITS

ABSTRACT – The aim of this study was to evaluate the effects of bulls' temperament kept at three different space allowances (6, 12, and 24 m^2/animal) on growth performance, carcass traits, meat quality traits and animal welfare. The specific objectives were: 1) to evaluate the effects of space allowance on bulls' temperament; 2) to evaluate the effects of temperament on growth performance, carcass and meat quality traits of beef cattle kept in different space allowance; 3) to evaluate the effects of temperament on adrenal gland morphometric and its relationship to space allowance; 4) to determine the relationship between adrenal gland morphometric and bruise severity on the carcass, at different space allowance. The study was conducted using 1,350 bulls (450 Nellore and 900 cross-bred Angus or Caracu x Nellore) raised on pasture and finished in a commercial feedlot. Temperament was assessed by the flight speed (FS) test on day 0 (FS_0), 35 (FS_{35}) and 87 (or last day; FS_{fin}) of the feedlot period. Feedlot growth performances, carcass quality traits and meat pH_{24}, adrenal gland morphometry and severity of bruises in carcasses were assessed. Flight speed decreased over time ($p < 0.01$) during the feedlot period. Overall FS neither differed between bulls kept at different space allowances ($p = 0.79$) nor between breeds ($p = 0.16$). The interaction between space allowance and breed or between FS_0 and FS_{fin} had no influence on any performance or carcass quality traits ($p > 0.05$). However, calm bulls according FS_{fin} had greater average daily gain from day 0 to 35 ($p < 0.0001$), were heavier on the 35th ($p < 0.0001$) and on the 87th ($p < 0.01$) day in the feedlot and, consequently, produced heavier carcasses ($p < 0.01$) than intermediate and reactive ones, which did not differ between them. Regardless of space allowance or breed, reactive bulls according to FS_0 had heavier adrenal glands ($p < 0.01$) and with greater perimeters ($p = 0.01$) when compared to the calm ones. Additionally, when kept in reduced space allowance (6 or 12 m^2/animal) reactive bulls had greater cortical area ($p < 0.05$) than intermediate and calm ones. In relation to severity of bruises, bulls with higher medullar area, greater perimeter and heavier of adrenal glands ($p < 0.05$) kept at 6 or 24 m^2/animal exhibited more severe bruising of the carcasses. We conclude that the feedlot conditions applied in this study improved bull temperament over time, and that, regardless of the space allowance provided to them and the breed, calm bulls showed better performances than intermediate and reactive ones. The animal ability to adapt to an intensive production system, such as feedlot, seems to contribute favorably to the quality of the production. The reduced space allowance in feedlots resulted in more detrimental effects on the welfare of reactive bulls than of the calm ones. Furthermore, it was clear that more stressed animals were more likely to have severe bruise on their carcasses.

Key words: environment, feedlot, flight speed, reactivity, Zebu cattle.

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CAPÍTULO 1 - Considerações gerais

1. Introdução

No Brasil, país detentor de posição de destaque no cenário global de produção e exportação de carnes, tem sido notório a crescente intensificação da bovinocultura de corte, principalmente pela utilização de confinamentos para a terminação de bovinos. O confinamento minimiza os efeitos da baixa disponibilidade de forragem no período seco do ano (MILLEN et al., 2009), além de ser uma das estratégias para aumentar a produtividade por área, atendendo a crescente demanda externa por carne bovina de qualidade (OLIVEIRA; MILLEN, 2014).

Confinar, de acordo com a premissa básica dessa atividade, implica na redução do espaço disponível por animal. Apesar dos confinamentos no Brasil apresentarem ciclos curtos entre 70 e 90 dias (MILLEN et al., 2009), estudos recentes tem demonstrado que a redução no espaço disponível, em confinamento ao ar livre (MACITELLI, 2015), similarmente aos confinamentos “indoor” (FISHER et al., 1997; HICKEY et al., 2003; GUPTA et al., 2007; AVERÓS et al., 2014), tem impacto negativo no desempenho, no comportamento, no ambiente e na saúde dos bovinos. Adicionalmente, a redução de espaço tem efeito sobre a morfometria das glândulas adrenais, que tem sido usada como indicador de estresse (SHIVELY; KAPLAN, 1984; ULRICH-LAI et al., 2006). Em conjunto, os resultados mencionados anteriormente, evidenciam um maior custo biológico durante o processo de adaptação do animal (BROOM, 1986; FRASER et al., 2013) quando submetido ao ambiente de confinamento com alta densidade. Além disso, é também reconhecido pelos consumidores que a restrição do espaço disponível para os animais em sistemas intensivos de produção tem impacto negativo no bem-estar dos animais e, consequentemente, na imagem do produto (VANHONACKER et al., 2009).

Nesse contexto, ressalta-se que as variações na percepção e nas respostas dos bovinos às situações desafiadoras caracterizam as diferenças individuais na susceptibilidade ao estresse e uma das formas de analisar essas diferenças individuais é pela avaliação do temperamento dos animais (KILGOUR et al., 2006;

KING et al., 2006). Bovinos reativos apresentam elevada concentração basal sanguínea de cortisol, lactato, ácido graxo não esterificado e glicose quando comparado aos animais calmos (CAFE et al., 2011a), indicando que existem diferenças intrínsecas tanto na resposta do eixo hipotálamo-hipófise-adrenal (CURLEY JR et al., 2006; KING et al., 2006; BURDICK et al., 2010), quanto do sistema simpático adreno-medular (CAFE et al., 2011a).

Ademais, devido a sua reação comportamental exacerbada quando abordados pelo homem, os bovinos reativos (FORDYCE et al., 1985), se comparado aos calmos, apresentam maior risco de acidentes (PARANHOS DA COSTA et al., 2000), maior probabilidade de ações agressivas pelos manejadores (RUEDA et al., 2015), bem como maior risco de serem transferidos para as baias hospitais do confinamento, principalmente em decorrência da inapetência e perda de peso, sem nenhuma causa clínica associada (FELL et al., 1998; 1999), o que sugere dificuldades adaptativas ao sistema.

Entretanto, ainda há pouca informação sobre os efeitos do confinamento sobre o temperamento de bovinos, e muitas delas são contraditórias. Por exemplo, Fordyce et al. (1985) e Turner et al. (2011) relataram que bovinos criados em sistemas mais intensivos são menos reativos ao manejo do que aqueles mantidos em sistema de criação extensiva, devido a maior frequência de manejo pelo homem ocorrendo habituação. Entretanto, os resultados de Burrow e Prayaga (2004) indicam que esse nem sempre é o caso.

Assim, parece razoável supor que bovinos reativos irão apresentar maiores dificuldades em enfrentar o ambiente do confinamento, o que pode ser potencializado pela redução do espaço disponível por animal, resultando em efeitos deletérios no desempenho, na qualidade das carcaças e da carne.

Diante do exposto, o objetivo dessa tese é avançar no conhecimento das questões mencionadas acima, avaliando os efeitos do temperamento no desempenho, na morfometria da glândula adrenal e, na qualidade da carcaça e da carne de bovinos mantidos em três espaços disponíveis (6, 12 e 24 m^2 por animal) nas baias de confinamento durante o período de terminação.

2. Revisão de literatura

2.1. A bovinocultura de corte brasileira em direção à intensificação

O Brasil possui o maior rebanho comercial do mundo com 212,34 milhões de bovinos (IBGE, 2014) e é detentor da segunda posição no ranking de exportação de carne bovina, após perder a liderança para a Índia (USDA, 2015a). Observa-se redução de 10% em volume e 17% em faturamento das exportações de carne bovina em 2015 comparada ao ano anterior (ABIEC, 2015b). Entretanto, espera-se rápida recuperação desse cenário na próxima década, com crescimento de 37,4% nas exportações de carne bovina (de 2,1 para 2,9 milhões toneladas) (MAPA, 2015a), impulsionada pela desvalorização da moeda brasileira e pela maior demanda de consumo na Ásia (USDA, 2015b).

Em relação à produtividade, o Brasil ainda é superado pelos Estados Unidos, líder no ranking de produção de carne bovina, principalmente devido à alta taxa de desfrute do rebanho, obtida pelo elevado número de animais confinados (USDA, 2015a). Projeções da Associação Brasileira das Indústrias Exportadoras de Carne (ABIEC, 2015) indicam que até 2020, o Brasil assumirá a liderança do ranking. Apesar da atual crise financeira, o incremento em produtividade esperado é condizente com o crescimento de 17,8% no consumo de carne bovina (de 7.2 para 8.5 milhões toneladas) para a próxima década no Brasil (MAPA, 2015a).

O Brasil tem sido apontado como o principal país em condições favoráveis em atender a crescente demanda mundial por carne bovina (OECD-FAO, 2015). Entretanto, tem sido proposto que a atual pecuária brasileira, em sua maioria caracterizada por sistemas extensivos com animais zebuínos e suas cruzas, em solos de baixa a média fertilidade no bioma Cerrado, com gramíneas africanas adaptadas (*Brachiaria spp.* e *Panicum spp.*) (FERRAZ; FELICIO, 2010) e com 30 milhões de hectares de áreas de pastagens em algum estágio de degradação (MAPA, 2015b), passará por mudanças nos próximos anos, em direção à intensificação do sistema de produção (MILLEN et al., 2011; MILLEN; ARRIGONI, 2013; RASMUSSEN et al., 2014).

Já há evidências de que essas mudanças estão ocorrendo, como caracterizado pelo aumento de 30% na taxa de desfrute do rebanho brasileiro em comparação aos 4% previstos para o crescimento do rebanho na última década (MAPA, 2015a). Em parte, esse incremento em produtividade pode ser atribuído ao crescente uso dos confinamentos, pois de acordo com a Associação dos Confinadores (ASSOCON, 2015), observou-se crescimento exponencial do número de bovinos terminados em confinamento entre 2010 (2,0 milhões de bovinos) e 2014 (4,5 milhões de bovinos). E para 2023, espera-se que 9,0 milhões de bovinos sejam terminados em confinamentos (RASMUSSEN et al., 2014).

Confinar bovinos no Brasil, inicialmente, restringia-se ao fato de favorecer a produção de carne durante a estação seca, período de menor disponibilidade de forragens e maior preço da arroba (MILLEN et al., 2009). Entretanto, observa-se expansão dessa atribuição inicial, onde a utilização estratégica dos confinamentos tem sido associada com a redução do ciclo de produção (MILLEN et al., 2011), da pressão sobre os recursos naturais (CEDERBERG et al., 2009; PELLETIER et al., 2010), da competição das culturas de grãos por área de terras agrícolas (RASMUSSEN et al., 2014), bem como ao favorecimento da produção de carne de qualidade constante ao longo do ano (MILLEN et al., 2009; OLIVEIRA; MILLEN, 2014).

2.2. A disponibilidade de espaço nos confinamentos e seus efeitos deletérios

Apesar dos evidentes benefícios produtivos e econômicos, o bem-estar de bovinos confinados é reduzido quando comparado ao de bovinos mantidos em pastagens (LEE et al., 2013), possivelmente pelo fato de sistemas intensivos não atenderem as necessidades básicas de bovinos (RUSHEN; DE PASSILÉ, 1992; BROOM; FRASER, 2007; PHILLIPS, 2008). De acordo com Vanhonacker et al. (2009), a redução de espaço disponível e o tamanho das baias são os principais fatores, atribuídos pela sociedade, para a limitação de bem-estar animal no confinamento.

Os produtores rurais, embora compartilhem da mesma percepção acerca da restrição de espaço (VANHONACKER et al, 2008), enfrentam conflitos econômicos advindo dessa percepção, uma vez que o espaço disponível ideal a partir da perspectiva do bem-estar animal é maior do que o mínimo necessário para a maximização dos lucros no confinamento (VANHONACKER et al., 2009). Assim, a partir de determinado ponto na escala, uma relação de não linearidade entre produtividade e bem-estar animal pode ser vista dentro dos sistemas produtivos, como reportado por McInerney (2004).

Apesar disso, o bem-estar de animais de produção está diretamente relacionado à qualidade dos produtos de origem animal (BLOKHUIS et al., 2003 e 2008; DE PASSILLÉ; RUSHEN, 2005), principalmente nas sociedades onde sustentabilidade e ética são o centro das discussões políticas e públicas sobre os sistemas de produção (VERMEIR; VERBEKE, 2006). No Brasil, ainda têm-se mínimo impacto econômico das questões éticas no ambiente de criação dos animais, embora um crescente interesse das cadeias produtivas de carne pela produção sustentável nos seus programas de controle de qualidade bem como na promoção do bem-estar animal e humano têm sido observado (PARANHOS DA COSTA et al., 2012).

Transferir animais para o ambiente de confinamento implica na imposição de diversos fatores ambientais como: mudança na dieta e no regime alimentar, reagrupamento social, maior exposição aos patógenos bem como exposição a condições climáticas extremas (FELL et al., 1998; MADER, 2003). Esses fatores estimulam a ativação de mecanismos adaptativos, em busca da manutenção da homeostase (MORMÈDE et al., 2007), ocasionando mudanças metabólicas, endócrinas e comportamentais que podem ser vistas ao longo do tempo de confinamento. Como descrito por Veissier e Boissy (2006), essa situação, se persistente, estabelece-se em posição oposta à definição operacional de bem-estar animal proposta por Broom (1986), uma vez que as muitas tentativas em adaptar-se ao ambiente de confinamento implicam em elevados custos biológicos para o animal.

Nesse contexto, a quantidade de espaço disponível por animal nas baias de confinamento parece ter papel fundamental em estimular ou minimizar essa dinâmica adaptativa. Uma grande variedade de espaço, 1,2 até 33 m^2 por animal, tem sido descritos na literatura (INGVARTSEN; ANDERSEN, 1993; GUPTA et al., 2007; WECHSLER, 2011; MADER, 2011). Entretanto, permanece inconclusivo qual seria a condição ótima em relação ao espaço disponível (em m^2) para bovinos confinados. Equações alométricas, baseando-se na forma e no tamanho do animal, providenciam o espaço necessário para os movimentos de levantar-se e deitar-se bem como a alternância entre ambos (PETHERICK et al., 2007; PETHERICK e PHILLIPS, 2009). Apesar disso, a complexidade das relações sociais e do repertório comportamental dos animais, como descrito por Keeling (1995), trazem dificuldades no estabelecimento do espaço que atenda as necessidades física e psíquica dos animais.

Do ponto de vista de desempenho, bovinos confinados em reduzidos espaços disponíveis apresentaram redução da taxa de ingestão, da eficiência alimentar e do ganho de peso (INGVARTSEN; ANDERSEN, 1993; FISHER et al., 1997; FELL et al., 1999; HICKEY et al., 2003; GUPTA et al., 2007). Em relação à saúde, maior ocorrência de espirro, tosse, corrimento nasal e ocular bem como achados macroscópicos *post-mortem* indicativos de pneumonia são encontrados devido as piores condições ambientais, caracterizada pelo maior acúmulo de lama e poeira nas baias de confinamento (MACITELLI, 2015).

Já do ponto de vista comportamental, aumento da frequência de interações agonísticas (KONDO et al., 1989; FISHER et al., 1997; KRAWCZEL et al., 2012) tem sido descritas em função dos conflitos sociais originados pela impossibilidade de fugir frente a um encontro competitivo e de manter seu espaço individual sem violação (FRASER, 1980; LINDBERG, 2001). Ainda, observa-se redução no tempo de permanência deitado (FISHER et al., 1997; NAPOLITANO et al., 2004; GYGAX et al., 2007), bem como no tempo de ruminação (FISHER et al., 1997).

2.3. Variação individual na adaptação à restrição de espaço no confinamento

Apesar dos resultados mencionados anteriormente serem apresentados no contexto de grupo como unidade experimental ou observacional, evidências sugerem que a variação individual tem importante efeito sobre as estratégias utilizadas nas tentativas de adaptar-se ao ambiente (KOOLHAAS et al., 1999, 2008 e 2010). Essa variação individual relaciona-se com a avaliação cognitiva do estímulo pelo animal (KOOLHAAS, 1999) e origina-se na plasticidade das respostas comportamentais (RUSHEN, 2000; FAUSTINO et al., 2015), fisiológicas (MASON, 1971) e neuroendócrinas (KOOLHAAS et al., 2010).

Em bovinos, o temperamento é uma importante característica do indivíduo relacionada à percepção e consequentemente no desencadeamento de respostas comportamentais e fisiológicas em diferentes contextos, sendo considerado um potencial indicador da capacidade de adaptação dos bovinos ao sistema de confinamento (FELL et al., 1999; BEHRENDS et al., 2009; SOARES, 2015). Entretanto, nenhum estudo até o momento tem avaliado o efeito do temperamento de bovinos em diferentes disponibilidades de espaço no confinamento.

2.4. Temperamento como fonte de variação individual nos sistemas de produção

2.4.1 Definição de temperamento

Temperamento pode ser caracterizado pela reação do animal aos humanos em uma dada situação (BURROW, 1997), sendo caracterizado principalmente pela avaliação das respostas comportamentais evocadas pelo medo (FORDYCE et al., 1982). Do ponto de vista prático, a facilidade ou a dificuldade com que é realizado o manejo dos animais durante as atividades de rotina na fazenda tem sido considerada um bom indicador do temperamento dos animais (MORRIS et al., 1994). Animais de bom temperamento são ditos como calmos enquanto os de pior temperamento são frequentemente descritos como agitados, reativos ou excitáveis (FORDYCE et al., 1985).

Evidências sugerem que o temperamento pode ser extrapolado para outros contextos além daqueles que envolvam o manejo pelo homem (PETHERICK et al., 2002; KILGOUR et al., 2006). Então, dentro do escopo dessa revisão de literatura, parte-se do pressuposto que temperamento não é meramente a resposta ao manejo pelo homem. Embora se reconheça que a definição operacional do termo proposta por Fordyce et al. (1982) e Burrow (1997) tenha aplicabilidade prática indiscutível e tenha guiado as pesquisas científicas promovendo avanços consideráveis no conhecimento do tema.

Tem sido amplamente reconhecido que temperamento é uma característica multifatorial (ROTHBART et al., 2000; GOSLING, 2001; GRAUNKE et al., 2013).

Com o intuito de categorizar os fatores que afetam o temperamento, Réale et al. (2007) propuseram cinco categorias importantes na expressão do temperamento, sendo elas: *i*) precaução/ousadia: reação do indivíduo em situações de risco que não envolvam novidade; *ii*) evitação/exploração: reação do indivíduo em situações que envolvam novidade; *iii*) atividade: nível de agitação do indivíduo em situações que não envolvam risco nem novidade; *iv*) agressividade: reação agonística do indivíduo contra membros da mesma espécie; *v*) sociabilidade: reação, com exceção das agonísticas, ao membros da mesma espécie.

2.4.2. Como medir o temperamento

Apesar da categorização apresentada, Paranhos da Costa et al. (2002) reforçaram a impossibilidade de definir uma única forma ou indicador capaz de medir o temperamento que integre todas as possíveis dimensões envolvidas na sua expressão. Ressalta-se que diferentes métodos de avaliação parecem medir diferentes aspectos do temperamento, visto pela baixa correlação entre eles, por exemplo, entre o teste de velocidade de saída e de escore de tronco (Kilgour et al., 2006), que tem sido atribuída ao fato de que provavelmente a velocidade de saída reflete um aspecto inato do temperamento enquanto que os escores de tronco relacionam-se com processo de aprendizagem (PETHERICK et al., 2002; MACKAY et al., 2013). Mas, até o momento, permanece inconclusivo qual aspecto do temperamento é avaliado em cada um dos testes.

Para ajudar a categorizar os métodos de avaliação do temperamento, Manteca e Deag (1993) propuseram uma divisão em três categorias: i) *testes comportamentais* (distância de fuga (FORDYCE et al., 1982); velocidade de saída (BURROW et al., 1988), escore de docilidade (LE NEINDRE et al., 1995), testes de novo objeto (FORKMAN et al., 2007) e teste de campo aberto (DE PASSILLÉ et al., 1995)); *ii*) *escalas com escores pré-definidos* (escore de tronco (VOISINET et al., 1997a,b; BURROW; CORBET, 2000; HOPPE et al., 2010); escore de movimentação (FORDYCE et al., 1988a; GRANDIN, 1993), escore de agitação no tronco de contenção (PARANHOS DA COSTA et al., 2002), escore de facilidade para contenção na pescoceira (HALL et al., 2011) e o escore de curral (BEHRENDS, et al., 2009)); *iii*) *escalas, sem escores pré-definidos, baseados na impressão do avaliador* (avaliação qualitativa do comportamento (SANT'ANNA; PARANHOS DA COSTA, 2013).

Dentre os listados anteriormente, os métodos mais comuns para avaliar o temperamento dos bovinos incluem o teste de velocidade de saída bem como os escores de tronco, ambos aplicados durante o manejo no curral (VETTERS et al., 2013; HASKELL et al., 2014). Para o contexto dessa revisão de literatura, serão descritos em detalhes os dois métodos mencionados acima, devido a sua aplicabilidade em um grande número de estudos, bem como sua confiabilidade com indicadores fisiológicos de estresse. Indivíduos reativos ou excitáveis, classificados por ambos os testes, apresentaram maior ativação do eixo hipotálamo-hipófise-adrenal (HHA) quando comparado com os animais calmos (CURLEY JR. et al., 2006; 2008; 2010; KING et al., 2006; PETHERICK et al., 2009; CAFE et al., 2011a).

Teste de velocidade de saída (VS): esta é uma das medidas mais utilizadas e conhecidas para avaliação do temperamento de bovinos de corte, sendo caracterizada pela objetividade e praticidade, com possibilidade de ser mensurada automaticamente por meio de dispositivos eletrônicos. O teste consiste em medir a velocidade (m/s) com que o animal sai do tronco de contenção em direção a um espaço aberto, geralmente uma das divisórias do curral (BURROW et al., 1988). Nesse teste assume-se que bovinos com menor VS são aqueles com melhor temperamento.

Teste de escore de tronco: variações na nomenclatura desse teste são apresentadas na literatura científica, embora todos eles sejam fundamentados na aplicação de notas para a reação dos bovinos durante uma determinada situação de manejo (TULLOH, 1961). Esses métodos avaliam o grau de perturbação ou de agitação dos animais contidos no tronco de contenção (ou na balança), atribuindo-se escores com base nos comportamentos dos animais quando mantidos presos no tronco, sendo que os valores extremos superiores representam animais reativos, enquanto valores extremos inferiores representam animais calmos (FORDYCE et al., 1982; GRANDIN, 1993). O método proposto por Fordyce et al. (1982) baseia-se na frequência e na intensidade de movimentação do animal dentro do tronco, variando de 1 a 7, e no grau de respiração audível dos animais, variando de 1 a 4. Grandin

(1993), também avaliando o nível de movimentação, propôs uma adaptação, redução de dois pontos na escala do método de Fordyce et al. (1982), guiada pela expectativa de que um método com sete escores seria difícil diferenciá-los na prática.

2.4.3 Fatores que afetam o temperamento

Interações entre fatores genéticos e ambientais têm sido descritas como capazes de afetar a formação e a expressão do temperamento dos animais ao longo do tempo (BURROW, 1997; GRANDIN; DESSING, 1998;). De maneira geral, considera-se que animais de raças zebuínas (*Bos taurus indicus*) são mais temperamentais que os de raças taurinas (*Bos taurus taurus*) (HEARNSHAW; MORRIS, 1984; VOISINET et al., 1997a; FORDYCE et al., 1988a; PIOVEZAN et al., 2013), sendo essas diferenças mais evidentes quando os animais são mantidos sob criação extensiva (BURROW; DILLON, 1997).

Entretanto, Burrow e Corbet (2000) verificaram que filhos de vacas Brahman com touros de raças continentais (Limousin e Charolês) foram mais reativos do que quando comparados com os filhos de touros britânicos (Angus, Hereford e Shorthorn), sugerindo que o Limousin e o Charolês combinaram efeitos negativos do temperamento quando cruzados com raça Brahman, ou simplesmente apresentaram piores temperamentos assim como os animais zebuínos. Resultados de estudos considerando as diferenças intrínsecas no temperamento dos animais baseados na composição genética merecem cautela em sua interpretação uma vez que fatores ambientais como a interação homem-animal e o sistema de criação são capazes de alterar o temperamento dos animais, tornando-os mais ou menos reativos (BOISSY; BOUISSOU, 1988; BECKER; LOBATO, 1997; SCHWARTZKOPF-GENSWEIN et al., 1997, COOKE et al., 2009; TITTO et al., 2010).

Como mencionado por Paranhos da Costa et al. (2002), a frequência e o tempo dispendido na interação homem-animal tem influência direta do sistema de criação utilizado, existindo uma expectativa de que os sistemas intensivos permitam uma maior frequência de interação entre humanos e bovinos, promovendo habituação (FORDYCE et al., 1985; TURNER et al., 2011). Entretanto, resultados opostos são apresentados por Burrow e Prayaga (2004) onde animais com temperamento reativo ou excitável mantido em sistemas intensivos, exacerbam os efeitos negativos do seu temperamento. Em sistemas extensivos com alta frequência de interação com os humanos em função do manejo de rotação de piquetes, do manejo de pesagem no curral ou durante o oferecimento de suplemento mineral, mudanças positivas no temperamento foram reportadas por Hoppe et al. (2010), Ceballos (2014), Góis (2014).

Diante desse contexto, podemos supor que a quantidade e a qualidade da interação homem-animal, apresentam-se como um melhor indicador para avaliar as consequências no temperamento dos animais do que somente o sistema de produção. Isso se deve pelo fato de que interações negativas reforçam a expressão das respostas comportamentais e fisiológicas desencadeadas pelo medo nos animais (GRANDIN, 1997).

2.4.4 Efeitos do temperamento no desempenho

O temperamento de bovinos tem efeito direto no ganho de peso, onde animais calmos ganham mais peso que os reativos (FELL et al., 1999; PETHERICK et al., 2002; BEHRENDS et al., 2009; SEBASTIAN et al., 2011). Apesar desses efeitos diretos, alguns estudos reportaram nenhum ou pouco impacto do temperamento nas características de crescimento de bovinos (BURDICK et al., 2009; HALL et al., 2011; SANT'ANNA et al., 2012).

Resultados distintos também são reportados para associação genética entre VS e ganho de peso, e variaram de r_g = 0.00 (BURROW, 2001; PRAYAGA e HENSHALL, 2005) até r_g = 0.58 (HOPPE et al., 2010). Por outro lado, VS apresenta correlação genética moderada com a taxa de conversão alimentar (r_g= 0.40 ± 0.26) e de consumo alimentar residual (r_g= - 0.59 ± 0.45), embora a correlação com a ingestão de matéria seca seja fraca (r_g= - 0.11 ± 0.26) (NKRUMAH et al., 2007).

Fenotipicamente, a VS foi correlacionada com a ingestão de matéria seca (r_f = - 0.35; NKRUMAH et al., 2007), entretanto nenhuma correlação foi reportada para a eficiência alimentar (kg de matéria seca/ kg de ganho) e o consumo alimentar (kg de matéria seca.dia^{-1}) (NKRUMAH et al., 2007; CAFE et al., 2011a). Para bovinos da raça Brahman, para cada 1 m/s de aumento na VS dos animais do tronco de contenção, espera-se redução de 20 kg no peso vivo final, 370 $g.dia^{-1}$ no consumo de matéria seca e 4,7 $min.dia^{-1}$ no tempo despendido no cocho (CAFE et al., 2011b).

Adicionalmente, animais reativos têm suas reações motivadas principalmente pelo medo, expressando comportamentos de alerta por longo tempo, o que reflete no incremento energético de mantença e na eficiência alimentar afetando negativamente o ganho de peso, como reportado por Petherick et al. (2002). Nessa linha de raciocínio, pesquisas apontam evidências (CURLEY JR. et al., 2010; CAFE et al., 2011a) que uma intrínseca dissimilaridade tanto na ativação como na resposta dos eixos HHA e do sistema simpático adreno-medular (SAM) têm sido utilizadas para explicar as diferenças encontradas entre o temperamento de bovinos, como apresentado a seguir.

2.4.5 Relação entre temperamento e dinâmica dos eixos hipotálamo-hipófise-adrenal e sistema simpático adreno-medular

O medo, principal desencadeador de respostas que caracterizam em parte o temperamento de bovinos, tem sido descrito como um importante estímulo na ativação do eixo HHA, desencadeando uma cascata de respostas endócrinas. Inicia-se pelo aumento da liberação do hormônio liberador de corticotrofina (CRH) do núcleo para-ventricular do hipotálamo, o qual age na hipófise anterior, liberando o hormônio adrenocorticotrófico (ACTH), que estimula o córtex da glândula adrenal a sintetizar e liberar cortisol para a manutenção da homeostase metabólica. Assim, o cortisol pode ser um indicador fisiológico da resposta ao estresse ou ser indicativo do nível de susceptibilidade do indivíduo ao estresse, sendo considerado fundamental para a investigação da função do eixo HHA (SAPOLSKY et. al., 2002).

Bovinos reativos tem uma maior concentração de cortisol se comparados aos calmos (CURLEY JR. et al., 2006 e 2008; PETHERICK et al., 2009; CAFE et al., 2011a). Entretanto, as diferenças encontradas entre os temperamentos não permanecem estáveis após os desafios com o CRH, o ACTH e a vasopressina (VP) exógenos (CURLEY JR. et al., 2008; 2010; CAFE et al., 2011a). Adicionalmente Cafe et al. (2011a) sugeriram que as diferenças no temperamento de bovinos também sofrem influência do SAM, onde no período anterior ao desafio com ACTH, novilhos reativos da raça Brahman, além da maior concentração de cortisol plasmático, apresentaram também maior concentração de glicose, lactato e ácido graxo não esterificado, se comparado aos calmos. Esses resultados sugerem ação da adrenalina e noradrenalina ou de ambas, em resposta ao manejo inicial pelo homem ou em ações permissivas combinadas com cortisol para manter a homeostase durante situações estressantes.

De fato uma expressiva diferença na concentração de cortisol entre os bovinos reativos e calmos, ao longo de todo o período anterior ao desafio com CRH, ACTH ou VP como mencionado acima, indica uma contínua dissimilaridade na resposta da adrenal a situações estressantes, sugerindo uma diferença intrínseca na função basal do eixo HHA entre os bovinos reativos e calmos. Mas, os resultados após o desafio sugerem que possivelmente ocorreu habituação, com redução da resposta da hipófise e da glândula adrenal ao longo do tempo. Apesar dessa constatação, as comparações da área sob a curva de resposta e o tempo para retornar as concentrações basais de cortisol demonstraram uma resposta moderada da adrenal nos animais reativos.

Como comentado por Curley Jr. et al. (2010), os resultados advindos de estudos que investigam a função do eixo HHA e do temperamento devem sempre levar em consideração os efeitos dos procedimentos experimentais relacionados as coletas de sangue bem como do modelo de temperamento bovino que envolve um subconjunto de animais previamente selecionados pela sua maior capacidade de resposta ao manejo pelo homem. Apesar dessa ressalva, até o momento, a questão que permanece inconclusiva e tem intrigado os pesquisadores é se a elevada concentração de cortisol no período anterior ao desafio hormonal é efeito do estresse agudo causado, por exemplo, pelo manejo pelo homem ou reflete um estado de estresse crônico (CURLEY JR. et al., 2008 e 2010; CAFE et al., 2011a).

Indicadores indiretos das alterações do eixo HHA e do SAM, poderiam contribuir para o melhor entendimento dessa questão. Evidências sugerem que a morfometria da glândula adrenal poderia ser um bom indicador indireto de estresse (SHIVELY e KAPLAN, 1984). O aumento de tamanho e peso da glândula adrenal tem sido descrito em ratos submetidos a protocolos de estresse crônico, onde se observa hipertrofia e hiperplasia na zona fasciculada da área cortical e hipertrofia na zona medular, associada com a diminuição no tamanho da célula na zona glomerular (ULRICH-LAI et al., 2006).

Yeakel e Rhoades (1941) relataram que o tamanho da glândula adrenal apresenta uma variação genética significativa entre linhagens de ratos caracterizados como ‘emocionais’ ou ‘não emocionais’, sendo as maiores glândulas encontradas na linhagem ‘emocional’, caracterizando animais mais propensos a reações de luta e fuga. Barnett et al. (1958) sugeriram que o temperamento dos ratos selvagens tem influência no tamanho da adrenal, onde características como agressividade ou excitabilidade levam a expressão de um comportamento que inevitavelmente colocam o indivíduo em situações estressantes, o que pode caracterizar um processo bidirecional, como reportado por Tyrka et al. (2008).

Ressalta-se que a interação genótipo-ambiente tem atividade controladora sobre a função dos eixos HHA e SMA e, obviamente fazem parte de uma dinâmica extremamente complexa. Como reportado por Macitelli (2015), a redução de espaço no ambiente de confinamento afetou a morfometria das glândulas adrenais, onde animais mantidos em espaços reduzidos, no momento do abate, apresentaram glândulas adrenais mais pesadas e com maior área cortical quando comparado a de animais mantidos em maior disponibilidade de espaço. Esses resultados corroboram com Friend et al. (1979), que associaram o estresse gerado pela restrição de espaço no confinamento de bovinos à maior atividade funcional das glândulas adrenais. Entretanto, o mesmo não tem sido reportado por Gupta et al. (2007) e Krawczel et al. (2012).

Como amplamente reconhecido, animais submetidos a estresse crônico tem seu desempenho afetado, uma vez que a elevada concentração de cortisol inibe diretamente a secreção do hormônio do crescimento, das gonadotrofinas e da tirotrofina, fazendo com que os tecidos alvos dos esteroides sexuais e dos fatores de crescimento fiquem resistentes a esses hormônios, devido à ação antagônica do cortisol, impedindo a lipólise e o anabolismo dos músculos e ossos. Apesar de o cortisol promover um aumento da glicose disponível para o organismo, a mesma não é eficientemente utilizada pelos tecidos periféricos, devido à sensibilização das células à insulina provocada indiretamente pelos próprios hormônios do estresse

(HABIB et al., 2001; UCHOA et al., 2014), sendo a eficiência de utilização da energia reduzida. Fell et al. (1999) reportaram correlação negativa (r = - 0.54) entre a concentração de cortisol e o ganho de peso. Adicionalmente, não só o ganho de peso dos animais pode reduzir quando um animal é submetido a estresse crônico, mas também a composição do ganho de peso pode ser alterada, principalmente pelo evidente aumento da distribuição do tecido adiposo, principalmente na cavidade abdominal (SHIVELY et al., 2009).

Para finalizar, o modelo de temperamento bovino pode ser útil para decifrar os mecanismos associados à hiperatividade do eixo HHA, elucidando fenótipos fisiológicos ou patologias que têm paralelos em outras espécies, como por exemplo, a depressão maior e as síndromes relacionadas à ansiedade, em humanos (RUBIN et al., 1996; SZIGETHY et al., 1994; RISBROUGH; STEIN, 2006).

2.4.6 Relação entre temperamento e qualidade de carcaça e da carne

Como apresentado no item anterior dessa revisão, evidências sugerem que o temperamento dos bovinos está intimamente associado com a susceptibilidade ao estresse (CURLEY JR. et al., 2006; 2008; KING et al., 2006; TITTO et al., 2010). Durante as etapas do manejo pré-abate, conhecidamente como fontes inevitáveis de estresse (FERGUSON; WARNER, 2008), parece razoável assumir que animais de pior temperamento apresentarão aumento nos níveis de cortisol e de adrenalina (KING et al., 2006; BURDICK et al., 2010), com efeitos negativos direto na qualidade das carcaças e da carne (VOISINET et al., 1997b; PETHERICK et al., 2002; KING et al., 2006). Os principais efeitos relacionam-se com a maior ocorrência de hematomas nas carcaças e maior depleção do glicogênio muscular, resultando em carne DFD (do inglês “Dark, Firm and Dry”), considerados grandes problemas da indústria da carne tanto do ponto de vista de quantidade como de qualidade de carcaça e carne (FERGUSON; WARNER, 2008; SHOOK et al., 2008).

Cada hematoma na carcaça bovina pode ser definido como uma lesão traumática com injúria tecidual e ruptura do suprimento vascular (HOFFMAN et al., 1998), desenvolvido após aplicação de força suficiente para romper os vasos sanguíneos (BARICIAK et al., 2003), provocando hipersensibilidade e dor ao redor da área (GREGORY, 2004). Intuitivamente, poderíamos supor que bovinos reativos apresentam maior número de hematomas nas carcaças. Entretanto os resultados dos estudos envolvendo esse tema são inconclusivos, variando de nenhuma evidência científica de relação (FORDYCE et al., 1985; BURROW; DILLON, 1997) para aumento de 0.3 kg de hematomas retirados por carcaça a cada ponto aumentado no escore de temperamento, o que resulta numa diferença de 1.5 kg de hematomas, entre os mais reativos e os mais calmos (FORDYCE et al., 1988b).

Adicionalmente, foram encontrados efeitos do temperamento na localização dos hematomas nas carcaças, sendo as áreas do lombo, da tuberosidade coxal e da tuberosidade isquiática as mais acometidas, incluindo músculos adjacentes que originam os cortes cárneos de maior valor econômico incluindo os músculos *Longissimus dorsi*, *Glutaeus medius, Biceps femoris*, *Semimembranosus* e o *Semitendinosus* (FORDYCE et al., 1988b).

Ainda nesse contexto, Fordyce et al. (1985) sugeriram que a maior susceptibilidade dos bovinos na ocorrência de hematomas nas carcaças pode ser em função da micro anatomia e da fisiologia do músculo, combinadas com a fisiologia do estresse. Barnett et al. (1984) apresentaram interessantes resultados em relação a essa associação proposta por Fordyce et al. (1985), onde vacas em estado de estresse crônico apresentaram hematomas mais severos quando comparadas com as vacas sob estresse agudo ou do grupo controle. Uma menção nesse sentido se faz importante, Hamdy et al. (1957a,b) indicaram que a resposta do tecido da área com hematoma é afetado pela força mecânica aplicada bem como o tipo de evento que ocasionou o hematoma além da localização anatômica, onde áreas com protuberâncias ósseas e/ou bem vascularizadas produzem hematomas mais severos.

A presença de hematomas na carcaça está associada aos altos valores de pH final da carne (STRAPPINI et al., 2013, MCNALLY; WARRIS, 1996) e este, por sua vez, tem relação direta com a maior força de cisalhamento, afetando a maciez da carne (PURCHAS et al., 2002). Sabidamente, a maciez da carne tem uma variação genética inerente. Entretanto, o temperamento dos animais vem sendo descrito como um importante fator nessa dinâmica (VOISINET et al., 1997a,b), embora os resultados sejam contraditórios.

Voisinet et al. (1997b) relataram que bovinos reativos apresentaram maior proporção de carne de coloração escura quando comparados com os calmos, embora o pH final permaneceu entre 5.3 e 5.8, não sendo considerado alto o suficiente para caracterizar uma carne verdadeiramente DFD. Cafe et al., (2011b) também reportaram que animais reativos apresentaram coloração mais escura do músculo *Longissimus lumborum*, elevado pH final da carne, maior força de cisalhamento e maior porcentagem de perda por cocção. Em contraste, nenhuma associação entre temperamento e incidência de cortes escuros ou elevado pH final da carne foi encontrado nos estudos de Fordyce et al. (1985) e Petherick et al. (2002).

Evidências sugerem que o temperamento afeta a maciez da carne de bovinos (KING et al., 2006; BEHRENDS et al., 2009; DEL CAMPO et al., 2010; HALL et al., 2011; MAGOLSKI et al., 2013), com correlação genética fraca a moderada (r_g = 0.30 - 0.40; KADEL et al., 2006; REVERTER et al., 2003). De acordo com King et al., (2006) a maior susceptibilidade ao estresse dos animais reativos parece criar uma condição menos favorável para a proteólise mediada pelas calpaínas. Ainda, bovinos reativos apresentam alto grau de agitação, o que fisiologicamente poderia promover maior intensidade de contração das fibras musculares, provocando hipertrofia muscular bem como aumento do diâmetro das fibras musculares e encurtando dos sarcômeros, ocorrendo então à redução na maciez da carne. Apesar dessas pressuposições, Ferguson e Warner (2008) evidenciam que possivelmente outros mecanismos desconhecidos estão envolvidos nessa dinâmica. Contrariamente aos resultados apresentados, nenhuma associação entre temperamento e qualidade da carne foi encontrado por Fordyce et al. (1985; 1988b), Petherick et al. (2002) e Turner et al. (2011), sendo esses resultados atribuídos ao baixo nível de reatividade dos animais, bem como à baixa ocorrência de problemas de qualidade da carcaça e da carne nas condições avaliadas nos respectivos estudos.

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CAPÍTULO 2 - Effects of temperament on performance, carcass and meat quality traits of pure Nellore and cross-bred bulls kept in feedlot at different space allowances

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ABSTRACT - The aim of this study was to evaluate the effects of temperament on feedlot performance, carcass and meat quality traits of confined bulls raised at three different space allowances (6, 12, and 24 m^2/animal) in feedlot pens during the finishing period. The study was conducted using 1,350 bulls (Nellore purebred or crossbred) raised on pasture and finished in a commercial feedlot. Temperament was assessed by the flight speed (FS) test on day 0 (FS_0), 35 (FS_{35}) and 87 (or last day; FS_{fin}) of the feedlot period. Feedlot growth performances, carcass quality traits and pH_{24} in the *Longissimus dorsi* (LD) muscle were assessed. Flight speed decreased over time with higher ($p < 0.01$) speed being recorded on FS_0 followed by FS_{35} and FS_{fin} at each space allowance. Overall, FS neither differed between bulls kept at different space allowances ($p = 0.79$) nor between breeds ($p = 0.16$). The interaction between space allowance and breed or between FS_0 and FS_{fin} classes (FSC_0 and FSC_{fin}) had no influence on any performance or carcass quality traits ($p > 0.05$). However, calm bulls as assessed by FSC_{fin} had greater ($p < 0.05$) average daily gain from day 0 to 35, were heavier ($p < 0.05$) on day 35 and 87 and consequently produced heavier ($p < 0.05$) carcasses than intermediate and reactive ones. No effect of cattle temperament, space allowance or breed on LD muscle pH_{24} was found ($p > 0.05$). Based on the results of this study, it can be concluded that bulls with calm temperament present better growth performances, regardless of the raising conditions in the feedlot.

Keywords: carcass quality, feedlot, flight speed, growth, meat quality, Zebu cattle

1. Introduction

Temperament of cattle is expressed by their behavioral responses during handling and is usually assessed through standardized techniques that measure their levels of reactivity, fear, agitation and/or aggression towards humans (Fordyce et al., 1985; Burrow, 1997). One of the most common method to assess cattle temperament is the flight speed test (FS), which is applied during cattle handling in the corral (Vetters et al., 2013). Flight speed has been related to cattle susceptibility to stress during handling, with excitable individuals presenting greater activation of the pituitary and adrenal glands when compared to calmer ones (Curley Jr. et al., 2008; Cafe et al., 2011a).

It has been hypothesized that the greater levels of responsiveness to stress in cattle may lead to undesirable effects on their growth performance, and on carcass and meat quality (Cafe et al., 2011b). However, the results reported so far are inconsistent and range from no impact (King et al., 2006; Hall et al., 2011; Turner et al., 2011) to direct effects of cattle temperament on growth performance, such as weight gain and dry matter intake (Voisinet et al., 1997a; Nkrumah et al. 2007; Cafe et al., 2011b), carcass traits, such as hot carcass weight and fat coverage (Cafe et al., 2011b), and the production of dark cutters (Voisinet et al., 1997b).

A greater number of carcass bruises may be expected from flighty and agitated cattle than from quieter ones pre-slaughter. However, there is no scientific evidence reporting such relationship (Fordyce et al., 1985; Burrow and Dillon, 1997). The inconsistency in these results may be attributed to the differences in cattle genetics or raising conditions between the studies, with cattle raised in more intensive raising systems being less reactive to handling than those kept under extensive pasture-based systems, probably due to the more frequent handling and thus habituation to humans (Fordyce et al., 1985; Turner et al., 2011). Nevertheless, this seems to be not always the case, since opposite results were found by Burrow and Prayaga (2004), who reported that under more intensive systems the constant contact of cattle with humans may lead to more excitable temperament.

Within the effects of feedlot conditions on cattle temperament, it is unclear if, besides the effects of the frequent human contact, the different levels of intensification (or space allowance) applied for confined cattle during the finishing period may contribute to the variation in their temperament resulting in changes in their productive performance. Indeed, limited space allowance for confined cattle has been shown to be a major source of physical, environmental and social stress (Macitelli, 2015). Considering the lower ability to cope with stressful situations due to greater sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis (Curley Jr. et al., 2008), flighty and agitated individuals may hardly cope with the feedlot environment as the intensification increases, eventually resulting in lower performance and poor carcass and meat quality.

The aim of this study was to evaluate the effects of temperament on feedlot performance and carcass and meat quality traits of confined bulls raised at three different space allowances (6, 12, and 24 m^2/animal) in feedlot pens during the finishing period.

2. Material and Methods

2.1. Ethical approval of animal use

All experimental procedures performed in this study were approved by the Committee for Ethical Use of Animals (Certified n. 025961/13) of the Faculty of Agricultural and Veterinary Sciences of the São Paulo State University (Jaboticabal, Brazil).

2.2. Location and climate conditions

The experiment was conducted in a commercial feedlot (15º32'48" S and 55^{o}10'08" W) located in Campo Verde (MT, Brazil). Lot feeding was divided into a dry and a rainy period (August-September and October-November, respectively). During the dry period the average ambient temperature was 34°C (ranging from 27 to 37°C) and the average relative humidity was 38% (ranging from 23 to 66%). Whereas, during the rainy period, the average ambient temperature was 32°C (ranging from 30 to 37°C) and the relative humidity was 59% (ranging from 37 to 70%).

2.3. Animals, treatments and pre-slaughter conditions

The study was conducted using 1,350 bulls raised on pasture and finished in a commercial feedlot (87 days). Bulls were either Nellore purebred (n = 450) or crossbred (F1 Nellore x Angus and F1 Nellore x Caracu; n = 900). Bulls were transported at the age of 30±6 months and at a live weight of 392±46 kg from two different farms of origin to the feedlot facility (approx. 300 km trip), where they were kept on pastures of *Brachiaria ruzizienses*, with free access to mineral supplementation and water, for 15 days. On day 0 of the feedlot period bulls were weighed, dewormed and received clostridial vaccine. Bulls were then distributed by breed through 9 lots of 150 animals each, with three pens housing Nellore purebreds and six pens keeping Nellore crossbreds.

All feedlot pens were 50 m wide, but differed in length (18, 36 and 72 m), generating three space allowances (6, 12 and 24 m^2/animal). One group of Nellore purebred and two groups of crossbred were allocated within each space allowance. Round water troughs (~ 1.5 m diameter) were common for two pens. In all pens bulls had access to a 50 m feed bunk line (33 cm/animal). Two diets were used during the feedlot period: an “adaptation” diet, offered in the first 25 days, and a “finishing” diet, offered from the 26^{th} day until the end of the feedlot period. The diet composition is shown in Table 1. Animals were fed four times a day: at 07:30 am, 10:00 am, 2:30 pm and 4:30 pm.

Table 1. Composition of “adaptation” and “finishing” diets offered to the bulls during the feedlot period (calculated as % of dry matter)[a,b,c]

Ingredients	**Adaptation diet**	**Finishing diet**
Corn silage	44.03	14.00
Cotton hull	0.00	12.50
Corn residue[d]	7.09	10.20
Milled corn	26.18	40.30
Cotton seed cake	12.67	12.38
Soybean skin	8.13	8.84
Other[e]	1.90	1.78
Total	100.00	100.00

[a] Estimated dry matter intake = 2.4% of liveweight
[b] Total digestible nutrients (TDN) = 65% for adaptation diet and 72% for finishing diet
[c] Crude protein (CP) = 14% for adaptation diet and 18% for finishing diet
[d] Residue from corn storage
[e] 14.93% mineral salt, 85% urea and 0.07% Monensin sodium

Due to commercial feedlot conditions, the time of shipment to slaughter was decided by feedlot managers taking into account body weight, fat coverage and market price. At the end of the feedlot period, bulls were transported to a commercial slaughterhouse (12 ± 4 h trip) at a density of 0.54 m^2 per animal, as recommended by Paranhos da Costa et al. (2010).

On arrival at the slaughter plant (slaughter capacity of 1,000 animals per day and slaughter speed of 120 animals/h), bulls were kept in lairage for 28 h (± 4 h) and were handled using flags. Electric prod were only used when needed. Animals from different pens (or treatments) were never mixed during the marketing process (from farm to slaughter). Animals were restrained in a stunning box and stunned with a captive bolt (USSS – 1, Jarvis Products Corp., Middletown, CT) before exsanguination. All carcasses were electrically stimulated by applying a low voltage (12 V) on the hindquarter during the 10 s after exsanguination.

2.4. Assessment of temperament

Cattle temperament was assessed by the flight speed (FS) test at three different times. The first evaluation was done at the entrance of cattle into the feedlot (day 0) and was repeated on day 35 and on day 87 or last day in the feedlot prior to transport to slaughter). The FS test used in this study was an adaptation from the method described by Burrow et al. (1988). Briefly, the assessment was carried out by measuring the speed of each animal when exiting the crush after having been weighed. A stopwatch was used to record the time (s) taken by each animal to cover a distance of 1.76 m. This time was later converted into speed ($m.s^{-1}$). The animals exiting the crush at a faster speed were considered as having a more excitable temperament (Burrow et al., 1988). The differences between initial and final evaluation of FS (ΔFS) were used to assess the level of cattle habituation to handling by humans over the feedlot period. Animals with greater positive ΔFS values were considered to be more habituated to handling.

2.5. Feedlot performance and carcass and meat quality assessment

Feedlot performances were assessed by recording body weight (BW) on day 0 (BW_0), on day 35 (BW_{35}) and just prior to transportation to the slaughterhouse (BW_{fin}). Average daily gain (ADG, $kg*animal^{-1}*day^{-1}$) was calculated based on the following equation: $ADG = (BW_{fin} - BW_0)/\text{days in feedlot}$.

Immediately after slaughter, carcasses were halved and hot carcass weight (HCW) was measured before chilling at 1°C. Carcasses were visually graded by a trained technician by measuring the subcutaneous fat coverage (SFC) at the 6th, 9th and 12th ribs, on the dorsal side of the *Latissimus dorsi* and *Dorsal serratus* muscles and in the lower back and cushion region. To this end, it was used a 5-point scale, where 1 = absent or no visible fat; 2 = scarce or presence of little visible fat; 3 = median or reasonable presence of fat (approx. 3 to 6 mm thick); 4 = uniform or presence of fat (approx. 6 to 10 mm thick); 5 = excessive or presence of excess fat (> 10 mm thick; Brasil, 2004).

Bruises on the carcass were assessed after slaughter in a subsample of 150 carcasses within each breed and space allowance combination by counting 1) the number of total bruises with more than 2 cm of diameter; 2) the number of red or fresh and dark or old bruises (< 24 h and > 24 h old, respectively; Gracey et al., 1999) with more than 2 cm of diameter. Bruise seriousness (BSS) was assessed through a 1-9 scoring method based on the type of bruise (deep vs. superficial) and the size (> 10 cm wide bruise) at each anatomical location on the carcass, with the higher score indicating the greater negative effect on the carcass value (Table 2).

Table 2. Description of the score of bruises seriousness (BSS) applied during assessment of carcass on the slaughter line (AUSMEAT, 2011)

Position	Cut	Score	Number of serious bruises
Butt	Silverside or topside or thick flank	1	1
Rump	Rump	2	1
Loin	Loin	3	1
Forequarter	Blade or ribs or brisket	4	1
Hindquarter	One serious bruise each on any two of the hindquarter cuts specified in Scores 1, 2, 3	5	2
	One serious bruise each on any three of the hindquarter cuts specified in Scores 1, 2, 3	6	3
Hindquarter and Forequarter	One serious bruise on the forequarter plus one serious bruise on one only of the hindquarter cuts specified in Scores 1, 2, 3	7	2
	One serious bruise on the forequarter and one serious bruise each on any two hindquarter cuts specified in Scores 1, 2, 3	8	3
Side	One or more serious bruises each on the forequarter, buttock (silverside or topside or thick flank), hip (rump) and back (loin)	9	4

Meat quality was assessed by measuring pH at 24 h *post-mortem* (pH_{24}) in the *Longissimus dorsi* (LD) muscle at the interface between the 10^{th} and 11^{th} rib, using a portable pH meter with an automatic temperature compensation (Hanna HI 99163, Hanna Instruments®, Woonsocket, RI).

2.6. Statistical analyses

All statistical analyses performed in the current study were carried out using the Statistical Analysis Software (SAS Institute Inc., Cary, NC, 2002), and a probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests.

Spearman's rank correlation was used to analyze the relationships between FS test over time. To assess the effects of space allowance (6, 12, and 24 m^2) and breed (Nellore purebred and crossbred bulls) on FS measures, data were analysed using PROC MIXED in a repeated measure analysis over time. The model included space allowance, breed, time of evaluation in feedlot (day 0, 35 and 87) and all interactions as fixed factors. The effect of BW on day 0 (BW0) was fitted as a covariate in the whole model. No significant triple interactions were found between space allowance, breed and time of evaluation in the feedlot. An Heterogeneous Compound Symmetry (CSH) covariance structure was used for the analyses. To analyze ΔFS PROC MIXED with space allowance, breed and their interaction as fixed effect was used. Comparisons between the levels of space allowance were corrected by a Tukey adjustment.

Flight speed test values were categorized into three classes (FSC; C = calm, I = intermediate and R = reactive) using a criterion based on the mean ± 0.5 SD. The effects of temperament evaluated on day 0 (FSC_0) and on the final day (87) in the feedlot (FSC_{fin}) on performance variables (ADG, BW_{35} and BW_{fin}) and carcass and meat quality traits (HCW, SFC and pH_{24h}) were analyzed by PROC MIXED. The model included space allowance (6, 12, and 24 m^2), breed (Nellore purebred and crossbred bulls), temperament classes (FSC_0 and FSC_{fin}, analyzed separately), and all interactions as fixed factors. No significant triple interactions were found between space allowance, breed and temperament for all the analyses performed. The space allowance by breed interaction was used as the error term for testing space allowance and breed effects. The same model was used in the GLIMMIX procedure with a Poisson distribution to analyze the total, fresh and old bruises number as well BSS. Comparisons between the levels of temperament (calm, intermediate and reactive) were corrected by a Tukey adjustment.

3. Results

3.1. Effects of space allowance, breed and time in feedlot on bulls' temperament

Significant and positive correlations were found between FS_0 and FS_{35} (r = 0.36; $p < 0.001$; data not shown), FS_{35} and FS_{fin} (r = 0.50; $p < 0.001$; data not shown) and FS_0 and FS_{fin} (r = 0.47; $p < 0.001$; data not shown). Based on these relationships, bulls that had showed a greater FS_0 at the entrance into the feedlot kept on consistently showing it through the time in the feedlot (Table 3).

Table 3. Distribution (%) of flight speed classes (FSC: Calm, Intermediate and Reactive) on day 0 (FSC_0) and on the final day of the feedlot period (FSC_{fin})[1]

	FSC_{fin}			
FSC_0	Calm	Intermediate	Reactive	Total
Calm	19.96	10.08	2.52	32.56
Intermediate	16.18	16.57	7.56	40.31
Reactive	4.17	10.08	12.89	27.13
Total	40.31	36.72	22.97	100.00

[1] Flight speed test at day 0 and at day final were categorized into three classes (FSC0/fin: calm, intermediate and reactive) using a criterion on the mean ± 0.5 SD.

Furthermore, calm bulls presented a more stable temperament over the whole feedlot time (from FS_0 and FS_{fin}) compared with reactive and intermediate ones (Fig. 1). However, it is worth noting that a small proportion of bulls either turned from calm to reactive (7.74%) or viceversa (15.36%) in this study (Fig. 1).

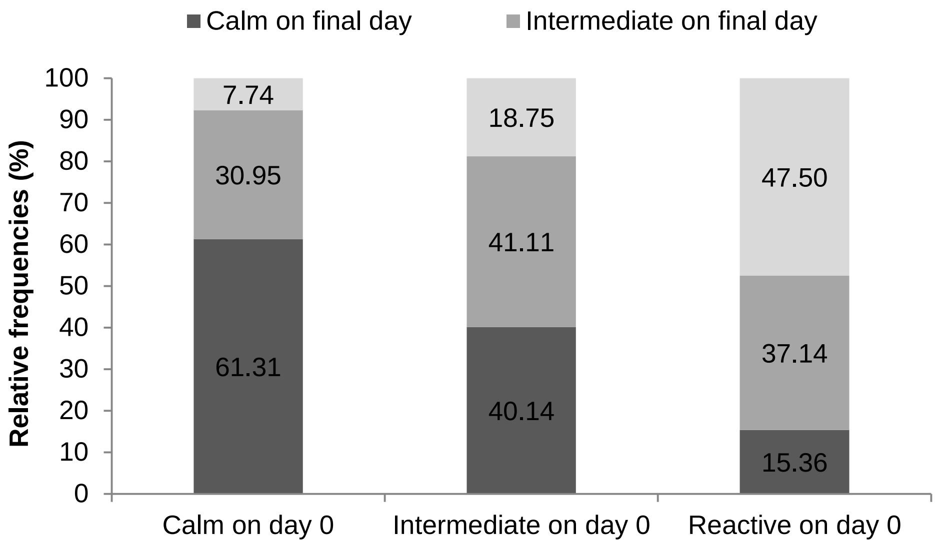

Figure 1. Proportion of bulls changing of temperament's classes according to FS test over the feedlot period (day 0 to final day)

Neither space allowance nor breed influenced the overall FS ($p = 0.79$ and $p = 0.16$, respectively; Table 4). However, FS decreased with the time spent in the feedlot (from FS_0 to FS_{fin}; $p < 0.01$). Space allowance and breed had no effect on ΔFS either ($p = 0.58$ and $p = 0.36$, respectively; Table 4). The interaction between space allowance, breed and time in feedlot neither influenced FS ($p = 0.73$).

Table 4. Least square means for flight speed (FS) test at each time of evaluation in the feedlot (Day 0, 35 and 87) and the difference between FS on day 0 and on the final day of the feedlot period (ΔFS) for each space allowance (SA)[1,2]

	SA6			SA12			SA24						
	Day in feedlot			Day in feedlot			Day in feedlot				*p-value*		
	0	35	87	0	35	87	0	35	87	SEM	SA	Breed[3]	TF[4]
FS	1.99^{a}	1.57^{b}	1.27^{c}	1.75^{a}	1.52^{b}	1.20^{c}	1.69^{a}	1.54^{b}	1.23^{c}	0.11	0.79	0.16	<0.01
	[1.56_2.43]	[1.14_2.00]	[0.84_1.70]	[1.32_2.18]	[1.09_1.95]	[0.77_1.64]	[1.26_2.12]	[1.11_1.97]	[0.80_1.66]				
ΔFS		0.73			0.53			0.46		0.17	0.58	0.36	--

[1] Within a row, means with a different superscript differ ($p < 0.01$) within each space allowance by Tukey adjustment

[2] SA6: 6 m^2/animal; SA12: 12 m^2/animal; SA24: 24 m^2/animal

[3] Breed: Nellore purebred vs. Nellore crossbred

[4] TF: time of feedlot

3.2. Effect of bulls' temperament on feedlot performance and carcass and meat quality traits

In this study, cattle that were assessed as calm on the last day of the feedlot period (FSC_{fin}) showed higher ADG_{0-35} ($p < 0.0001$), BW_{35} ($p < 0.0001$), BW_{fin} ($p < 0.01$) and HCW ($p < 0.01$) than intermediate and reactive ones (Table 5).

Table 5. Least square means of feedlot performance and carcass and meat quality traits for each class of temperament (C = calm, I = intermediate and R = reactive) on day 0 (FSC_0) and on the final day of the feedlot period (FSC_{fin})[1,2]

					p-value					
Variables[4]	C	I	R	SEM	SA	Breed	FSC	SA*Breed	SA*FSC	Breed* FSC
FSC0										
ADG_{0-fin}, kg/d	1.70	1.66	1.69	0.08	0.29	0.35	0.42	0.17	0.92	0.49
ADG_{0-35}, kg/d	2.09	2.05	2.02	0.21	0.83	0.65	0.33	0.80	0.51	0.02
ADG_{36-fin}, kg/d	1.48	1.49	1.50	0.10	0.21	0.20	0.90	0.37	0.84	0.91
BW_{35}, kg	463.92	462.45	461.37	12.86	0.89	0.54	0.38	0.86	0.55	0.03
BW_{fin}, kg	535.63	533.64	532.71	10.91	0.75	0.80	0.53	0.68	0.51	0.19
HCW, kg	296.50	295.26	296.03	5.46	0.94	0.66	0.68	0.77	0.09	0.44
SFC score	2.65	2.73	2.76	0.16	0.97	0.18	0.05	0.87	0.96	< 0.01
LD pH_{24}	5.69	5.70	5.70	0.02	0.89	0.21	0.21	0.80	0.69	0.52
FSCfin										
ADG_{0-fin}, kg/d	1.72	1.64	1.63	0.08	0.28	0.31	0.12	0.15	0.86	0.59
ADG_{0-35}, kg/d	2.15[a]	1.99[b]	1.88[b]	0.21	0.82	0.69	<0.0001	0.79	0.99	0.05
ADG_{36-fin}, kg/d	1.35	1.31	1.34	0.14	0.17	0.15	0.61	0.28	0.99	0.70
BW_{35}, kg	468.26[a]	462.57[b]	458.64[b]	7.11	0.88	0.57	<0.0001	0.85	0.99	0.05
BW_{fin}, kg	539.35[a]	532.60[b]	531.25[b]	12.00	0.78	0.82	< 0.01	0.67	0.99	0.56
HCW, kg	298.69[a]	294.48[b]	293.44[b]	5.14	0.95	0.61	< 0.01	0.79	0.56	0.66
SFC score	2.67	2.75	2.76	0.18	0.99	0.25	0.18	0.89	0.07	0.48
LD pH_{24}	5.68	5.70	5.70	0.02	0.92	0.22	0.20	0.77	0.65	0.60

[1] Within a row, means with a different superscript differ ($p < 0.05$) by Tukey adjustment
[2] SA: space allowance (6, 12 or 24 m^2 per animal); Breed: Nellore purebred vs. crossbred; FSC: Flight speed classes
[4] ADG0-35: average daily gain from day 0 to 35; ADG35-fin: average daily gain from day 35 to final; BW35: body weight on day 35; BWfin: body weight on the final day; HCW: hot carcass weight; SFC: subcutaneous fat cover score (1 = absent or no visible fat to 5 = >10 mm fat thickness; Brasil, 2004); LD: *Longissimus dorsi* muscle

Significant interactions were also found between breed and FSC_0 for ADG_{0-35} ($p = 0.02$), BW_{35} ($p = 0.03$) and for SFC ($P < 0.01$). When compared with calm and intermediate bulls, crossbred reactive bulls had lower ADG_{0-35} (C = 2.26 kg, I = 2.18

kg, R = 2.04 kg, SEM: 0.24, $p < 0.01$) and BW_{35} (C = 470.86 kg, I = 468.14 kg, R = 463.57 kg, SEM: 8.51; $p < 0.01$), but higher SFC score (C = 2.84, I = 3.02, R = 3.13, SEM: 0.19; $p < 0.0001$). No significant interaction ($p > 0.05$) between space allowance and breed or between space allowance and FSC (either FSC_0 or FSC_{fin}) were found for any growth performance or carcass and meat quality trait (Table 5).

Cattle temperament as assessed on day 0 (FSC_0) had an effect on BSS on the carcass, with calm and reactive bulls having higher ($p < 0.0001$) BSS than intermediate ones (Table 6).

Table 6. Least square adjusted means of total number of bruises, number of old and fresh bruises, and score of bruise seriousness (BSS) on the carcass for each class of temperament (C = calm, I = intermediate and R = reactive) as assessed on day 0 (FSC_0) and on the final day of the feedlot period (FSC_{fin})[1,2]

					p-value				
Variables[3]	C	I	R	SEM	SA	Breed	FSC	SA*FSC	Breed*FSC
FSC0									
Total	0.58	0.53	0.75	0.09	0.16	0.41	0.06	0.24	0.17
Old	0.17	0.15	0.21	0.04	0.33	0.67	0.50	0.82	0.60
Fresh	0.39	0.37	0.52	0.05	0.11	0.28	0.14	0.25	0.20
BSS	0.58[a]	0.33[b]	0.68[a]	0.09	0.25	0.20	<0.0001	0.16	0.89
FSCfin									
Total	0.63	0.63	0.67	0.06	0.09	0.15	0.89	0.03	0.02
Old	0.20	0.18	0.19	0.04	0.37	0.62	0.87	0.71	0.22
Fresh	0.42	0.44	0.46	0.06	0.07	0.16	0.91	0.08	0.09
BSS	0.47	0.40	0.49	0.08	0.19	0.24	0.43	0.01	< 0.01

[1] Within a row, means with a different superscript differ ($p < 0.05$) by Tukey adjustment
[2] SA: Space allowance (6, 12 or 24 m^{2}· animal); Breed: Nellore purebred vs. crossbred
[3] Total (superficial plus muscular; old plus fresh bruises on the carcass); Old (> 24 h old); Fresh (< 24 h old); BSS: bruise score seriousness (from 1 to 9; AUS-MEAT, 2011)

Significant interactions were found between SA and FSC_{fin} for the number of total bruises and BSS on the carcass. When compared with calm and intermediate bulls, the carcass of reactive bulls kept at a space allowance of 6 m^2/animal presented a greater number of total bruises (C = 0.53, I = 0.58, R = 0.84, SEM: 0.13; $p = 0.03$; data not shown). Whereas, a greater number of BSS was found on the carcass of calm bulls kept at 24 m^2/animal than on those from intermediate and reactive ones (C = 0.79, I = 0.43, R = 0.39, SEM: 0.18, $p = 0.02$; data not shown).

Significant interactions were also found between breed and FSC_{fin} for total number of bruise and BSS on the carcass, with the carcass of calm and reactive Nellore purebred bulls presenting a greater number of total bruises and BSS than that of intermediate ones (C = 0.85, I = 0.59, R = 0.77, SEM: 0.08; $p = 0.02$ and C = 0.40, I = 0.23, R = 0.54, SEM: 0.10; $p < 0.01$, respectively; data not shown).

4. Discussion

To our knowledge, this is first ever study that evaluated the effect of temperament on growth performance and carcass and meat quality traits of beef cattle kept in feedlot at different space allowances.

The FS measures obtained in this study were within the range reported in the literature for Zebu breeds and their crosses (Petherick et al., 2009; Sant'Anna et al., 2012). The significant positive correlations among FS over the feedlot period indicated a moderate level of individual consistency in this measure as previously reported (Burrow, 1997; Curley Jr. et al., 2006; Cafe et al., 2011b). However, in this study a small portion of bulls changed their FS over time, turning from calm to reactive, suggesting that the individual variation in the evolution of FS over time may be related to the ability of animals to adapt to the feedlot environment (Grandin, 1993; Behrends et al., 2009).

Besides being associated with innate fear response (Burrow, 1997; Petherick et al., 2002, 2003; Curley Jr. et al., 2006, 2008; Cafe et al., 2011a), FS may be also related with general agitation of animals (Kilgour et al., 2006; Petherick et al., 2009). In this study, FS was substantially faster on day 0 and slowed down over the lot-feeding period, regardless of breed and space allowance. This observation is similar to that reported in previous studies (Curley Jr. et al., 2006; Cafe et al., 2011b). The faster FS measured at the introduction of bulls into the feedlot in this study reveals their perception of novel human handling in the corral and the feedlot facilities as fear-eliciting stimuli. Whereas, the slower FS as assessed in the course of the feedlot period result from the animals' learning process and habituation to the human handling and feedlot environment. In this study, in fact, bulls were exposed to humans contact at least four times per day during feed bunk management for feed provision, once a week for the cleaning of the water troughs and were handled in the corral once a month. A number of studies, in fact, reported a reduced fear response to humans in cattle when the interaction involves food as they learn to associate human presence with food supply (Boivin et al., 1992; Munksgaard et al., 1997; Jago et al., 1999).

Another factor that may have contributed to the process of habituation is the predictability and controllability of the potential stressors (Hargreaves and Hutson, 1990), given that in our study the management of corrals were performed by the same stockpeople. However, it is also worth noting that the increasing liveweight (30% BW increase at the end of the feedlot period) and related lower agility (Petherick et al., 2002) may have contributed to the slower FS during and at the end of the feedlot period.

The lack of significant difference on FS on day 0 among space allowances reflects the uniformity of bulls' temperament at the beginning of the experiment as they all originated from pasture and had a similar previous experience with human during handling. However, the lack of effects of space allowance on FS on day 35 and on the last day on feedlot was unexpected as bulls kept at the highest density (6 m^2/animal) should have presented a faster FS than those kept at 12 or 24 m^2/animal due to a greater difficulty to cope with a novel challenging situation. In a recent study, in fact, the application of reduced space allowance in the feedlot negatively influenced growth performance and animal welfare (Macitelli, 2015).

Previous studies hypothesized that the measurement of cattle temperament early in the production cycle are more predictive of growth performance and carcass quality traits variation than later to overcome the bias of acclimation or habituation to handling procedures on the results (Petherick et al., 2002; Behrends et al., 2009). However, our results did not support this hypothesis, as the temperament of bulls as assessed at the entrance into the feedlot could not explain the variation in growth performance and carcass quality traits. Soares (2015) also failed to find such a relationship in Nellore cattle. However, in the present study, the temperament score obtained at the end of the feedlot period could explain the variation in final body weight and weight gain, with calmer bulls being 8 kg heavier at the end of the production cycle and producing a 5 kg heavier carcass than reactive bulls. Slower growth rates in reactive cattle as assessed by the FS test have been also reported in a number of other studies (Burrow and Dillon, 1997; Voisinet et al., 1997b; Fell et al., 1999; Behrends et al., 2009; Cafe et al., 2011b).

In this study, a reduced growth rate and body weight in the first 35 days of feedlot, and higher subcutaneous fat deposition in the carcass was only found in reactive crossbreds bulls compared with calm and intermediate individuals. These results are in agreement with previous studies that reported stronger phenotypic association between the temperament traits and ADG for European breeds and its crosses (Nkrumah et al., 2007; Hoppeet al., 2010) and no association for Nellore cattle (Sant'Anna et al., 2012, 2013).

The relationship between reactive temperament and lower feedlot performance reported in this study and in previous ones may be explained by the complex effect of temperament on the hypothalamic-pituitary-adrenal and sympatho-adrenal medullary system axis, resulting in greater energy requirements of reactive individuals than the calm ones (Rietema et al., 2015), regardless of the imposition or not of stressors (Curley Jr. et al., 2006, 2008; Cafe et al., 2011a). Behavioral responses of reactive animals could also explain their lower feedlot performance, because they consume considerable energy to maintain their state of high arousal and physical activity (MacKay et al., 2013) at detriment of the time that could be spent resting and ruminating (Petherick et al., 2000), while eating less. Greater FS has been, in fact, negatively related with dry matter intake (Nkrumah et al., 2007; Cafe et al., 2011b) and frequency of visits to the feed bunk (Soares, 2015).

Overall, in this study carcass bruises were mostly of “fresh type”, which means that they were more likely inflicted pre-slaughter. No effect of the breed was found on the number or seriousness of bruises in this study which is in contrast with previous studies reporting a greater number of bruises and bruise seriousness score in Nellore purebred than in crossbred bulls likely due to the lower easiness of handling (Hearnshaw and Morris, 1984; Fordyce et al., 1988; Voisinet et al., 1997a). A greater number of bruises was found in bulls scored as reactive at the end of the feedlot period and kept at 6 m^2/animal. Francisco et al. (2015) also reported an almost double number of total bruises on the carcass of reactive Nellore cattle compared with adequate (calm and intermediate) ones. The poor raising conditions in the feedlot may have worsened the already low ease of handling of reactive bulls observed in a companion study (Macitelli, 2015). However, a greater score for seriousness of bruises was recorded on the carcass of calm bulls kept at 24 m^2/animal in this study. This result is hard to explain. As reported by Fordyce et al. (1985) the behaviour of cattle during preslaughter handling is not only influenced by their temperament, but also by the way they are handled.

Overall, LD muscle pH_{24} values recorded in this study were within the acceptable range for good beef quality (Thompson, 2002) and were neither affected by cattle temperament nor by space allowance or breed. Literature results on the effects of animal temperament on meat pH are contradictory ranging from no effect (Fordyce et al., 1988; Petherick et al., 2002; del Campo et al., 2010; Francisco et al., 2015) to increased risk for dark cutters production in more reactive cattle (Cafe et al., 2011b). The lack of variation in pH_{24} values between categories of temperament and treatments may be either explained by the lack of variation in the dietary energy intake and the intensity and extent of stress exposure (McVeigh and Tarrant, 1982; Pethick and Rowe, 1996; Coombes et al., 2014). Both factors are known to influence muscle glycogen levels at slaughter and post-mortem muscle pH (Immonen et al., 2000; Knee et al., 2004).

5. Conclusions

The feedlot conditions applied in this study allowed an improvement in bull temperament over time, regardless of the space allowance provided and breed used, with calm bulls showing better productive performances than intermediate and reactive ones. However, our results related to the impact of space allowance and breed on growth performance, skin bruises and meat quality in cattle of different temperament are rather inconclusive, probably due to the limitation in applying a balanced distribution of treatments under commercial conditions due to market restrictions. Further research is needed to elucidate the effects of feedlot raising conditions on cattle temperament and their response to preslaughter handling.

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CAPÍTULO 3 - Temperament affects the adrenal morphometry and the severity of bruises on carcasses of bulls kept in the feedlot at three space allowances

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ABSTRACT - The aim of this study was to evaluate the effects of temperament on adrenal gland morphometry in confined cattle kept during finishing period at three different space allowances (6, 12, and 24 m^2/animal) and to determine the relationship between adrenal gland morphometry and the severity of bruises on carcasses. The study was conducted with 270 bulls (Nellore purebred and crossbred), raised on pasture and finished in a commercial feedlot. Temperament was assessed by the flight speed (FS) and reactivity (RE) tests on day 0 (FS_0, RE_0), and 87 or last day (FS_{fin}, RE_{fin}). The FS and RE tests were categorized in classes ($FSC_{0/fin}$; $REC_{0/fin}$: calm, intermediate and reactive). After slaughter, adrenal glands were collected measuring their cortical (ADCOR), and medullar (ADMED) areas, perimeter (ADPER) and weight (ADWEIGHT). The carcass bruises were recorded, assigning a composite score of bruises severity (SBS). Regardless of space allowance or breed, reactive bulls according to FS_0 had greater ADPER ($p = 0.01$) and ADWEIGHT ($p < 0.01$) compared with the calm ones. However, when kept in reduced space allowance (6 or 12 m^2/animal) reactive bulls according FSC_0 had greater ADCOR ($p < 0.05$) than intermediate and calm ones. According to REC_0 in the smallest space allowance (6 m^2/animal), reactive bulls had have greater ADPER and ADWEIGHT ($p < 0.05$) than calm and intermediate ones. Reactive bulls according to FSC_{fin} also had greater ADPER ($p = 0.01$) and a tendency for ADWEIGHT ($p = 0.10$) than calm ones, but no significant effect of REC_{fin} was found. In relation to carcass bruises, significant effects of ADMED, ADPER and ADWEIGHT were found for cattle kept in the smallest (6 m^2/animal) and largest space allowances (24 m^2/animal), with higher values of SBS for animals with the biggest glands ('high' classes). We concluded that reduced space allowance in feedlots resulted in more detrimental effects on the welfare of reactive bulls than of the calm ones. Furthermore, stressed animals were more likely to have severe bruise on their carcasses.

Keywords: animal welfare, feedlot, flight speed, reactivity, stress

1. Introduction

Cattle temperament has been operationally defined as behavioral responses to humans during handling, resulted from fear reactions (Fordyce et al., 1982, 1988). Research reports that cattle temperament is associated with a variable physiological response to stressful events, including intrinsic differences in hypothalamic pituitary adrenal (HPA) axis (Curley Jr. et al., 2006; King et al., 2006; Burdick et al., 2010) and in sympathetic adreno-medullary system (SAM) dynamics responses (Cafe et al., 2011a). Indeed, reactive cattle show a greater susceptibility to stress when compared to their calmer counterparts as indicated by the more elevated basal cortisol, lactate, non-esterified fatty acids and glucose concentration in blood (Cafe et al., 2011a). So far, it is still unclear whether this greater stress susceptibility results from the effects of a previous acute stressor related to human handling or from chronic stress response, which would explain the higher baseline cortisol concentrations in blood of reactive individuals (Curley Jr. et al., 2008 and 2010; Cafe et al., 2011a).

The stress response of cattle is often addressed using blood cortisol concentration, whose peaks within few minutes after the animal is exposed to a stressor, requiring blood sampling within 1 min after the contact with the animal (Hopster et al., 1999), which is unpractical in commercial operations. Additionally, evidences suggest that cortisol concentration is not a reliable indicator in a chronic stress response (Hemsworth and Barnett, 2000). Thus the adrenal gland morphometry emerge as a feasible indicator of chronic stress for cattle that will be slaughtered. Adrenal gland is, in fact, an important stress-responsive organ that is part of both, the HPA axis and SAM system, which increases in weight and size as response to chronic stress condition (Selye, 1973) due to cellular hypertrophy and hyperplasia in the cortical zone and the hypertrophy in the medulla zone (Ulrich-Lai et al., 2006).

Space allowance during the finishing period is one the most of important sources of stress in feedlot cattle (Fell et al., 1999; Gupta et al., 2007). Previous results from our research group (Macitelli, 2015) showed that bulls kept in feedlot at reduced space allowance (6 or 12 m^2 per animal) exhibited heavier adrenals with higher cortical zone area when compared with bulls kept at pens with 24 m^2 per animal. To our knowledge, no study has ever evaluated the variation in adrenal morphometry in cattle with different temperaments in response to different space allowances in the feedlot.

Welfare and economic implications may arise from the handling of chronically stressed feedlot cattle, especially the increased risk of bruises on carcass, resulted from the higher level of agitation and fear of animals during handling (Fordyce et al., 1985). As reported by Barnett et al. (1984) chronically stressed cows had significantly more severe bruised tissue than those acutely stressed what the authors attributed to the differences on cows' physiological state. However, this relationship has not been studied yet despite the importance of bruises for carcass downgrading and animal welfare impoverishment, being a recognized source of pain in cattle (Gregory, 2004).

Thus, the aims of this study were to evaluate the effects of temperament on adrenal gland morphometry in confined cattle kept at three different space allowances (6, 12, and 24 m^2 per animal) in feedlot pens during the finishing period; and to determine the relationship between adrenal gland morphometry and the severity of cattle bruises on the carcasses.

2. Material and Methods

2.1. Ethical approval of animal use

All experimental procedures performed in this study were approved by the Committee for Ethical Use of Animals (Certified n. 025961/13) of the Faculty of Agricultural and Veterinary Sciences of the São Paulo State University (UNESP, Jaboticabal, SP, Brazil).

2.2. Animals, treatments and pre-slaughter conditions

A total of 1,350 bulls were raised on pasture and finished in a commercial feedlot located in the Mato Grosso State (Brazil) (15º32'48" S and 55º10'08" W), from August to November (87 days feedlot period). The animals were kept in lots with 150 animals each, three of Nellore purebred and six Angus or Caracu x Nellore crossbred, and these lots were kept under three space allowances (6, 12 and 24 m² per animal). Feedlot pens had 50 m wide with 50 m feed bunk line (33 cm per animal), water was available at libitum all the time. The animals were feed with a total mixed ratio four times a day (07:30 am, 10:00 am, 2:30 pm and 4:30 pm), by offering an "adaptation diet" (with 65% of total digestible nutrients) during the first 25 days in the feedlot, and an "finishing diet" (with 72% of total digestible nutrients), from then to the their last day in the feedlot.

For this study we used 270 bulls, 100 of them Nellore purebred and 170 crossbred (F1 Angus or Caracu x Nellore). The sampled cattle were randomly chosen in the slaughter line, by sampling 20% of animals of each space allowance. The animals were transported (12 ± 4 h trip) to a commercial slaughterhouse, at a density of 0.54 m per animal according to recommendation of Paranhos da Costa et al. (2010). No mixing was done between animals from different pens during transport, lairage and pre-slaughter handling. Bulls were kept in lairage for around 28 h (± 4 h) and were handled easily, using electric prod only when extremely needed. All animals were restrained in the stunning box and stunned with captive bolt before exsanguination.

2.3. Assessment of temperament

Temperament was assessed twice, at the entrance of cattle into the feedlot (day 0), concurrently with handling in the corral for weighing, deworming and clostridial vaccination, and when they were weighed before loading (day 87 or day final) and being transported to the slaughterhouse.

Two temperament traits were assessed, the flight speed (FS) and reactivity (RE) tests. The FS test used in this study was an adaptation from the method described by Burrow et al. (1988), measuring the speed of each animal when exiting the crush after having been weighed. A stopwatch was used to record the time (s) taken by each animal to cover a distance of 1.76 m. This time was later converted into speed (m/s). The animals exiting the crush at a faster speed were considered as having a more excitable temperament (Burrow et al., 1988).

The RE test was adapted from the method, described by Fordyce et al. (1985) and Grandin (1993). It was performed by giving scores to the level of bulls movements (MOV) and tension (TEN) (Table 1) during the first 4 s after the entrance of bull in the squeeze chute, without using any restraint device (such as head bail and squeeze sides). Both scores were then summed (TEN + MOV) resulting in RE measurement, ranging from 2 to 9, and the animals with higher CS values were considered as those with more excitable temperament.

Table 1. Descriptions of the movement (MOV) and tension scores (TEN) applied during the crush score test.

Traits	Scores descriptions
MOV	1- no movement; 2- little movement, during less than half of the observation time, 3- frequent movements (during half of the observation time or more), but not vigorous; 4- constant and vigorous movements; 5- constant and vigorous movements, animal jumps and raises its forelimbs off the ground.
TEN	1- the animal did not exhibit sudden movements of the tail, head and neck, no muscle tremors and eye white was not visible; 2- the animal exhibited few sudden movements of the tail, head and neck, no muscle tremors, and eye white may have been visible or not; 3- the animal exhibited continuous and vigorous movements of the tail, head and neck, no muscle tremors, and eye white was visible; 4- the animal appeared paralyzed "freezing"; showing muscle tremors and visible eye white.

2.4. Assessment of the adrenal gland morphometry

The right and left adrenal glands were collected on the slaughter line, dissected, refrigerated at 4 ºC and taken to the laboratory, where they were weighed and frozen at -18 ºC pending analysis. The assessment of adrenal gland morphometry was adapted from Shively and Kaplan (1984). Briefly, adrenal glands were fixed in Bouin's solution for 24 h and sectioned by a mid-sagittal cut along the maximum diameter. The exposed sagittal plans were photographed three times with a digital camera maintaining the lens parallel to the surface. The software Image J© (version 1.48, NIH, Bethesha, MD, USA) and digitizer table CTH470L Bamboo® (Wacom, Kazo, Japan) were used to obtain the measurements of total and cortical areas, with the medullar area being calculated by difference between total and cortical areas. To correct these data for differences in weight body of animals, cortical and medullar areas, as well as the perimeter and weight of adrenal glands were adjusted for 100 kg of hot carcass weight, generating the following variables: cortical area (ADCOR, cm^2), medullar area (ADMED, cm^2), perimeter (ADPER, cm) and adrenal weight (ADWEIGHT, g).

2.5. Assessment number and severity of bruises on the carcasses

The number of bruises per carcass was recorded. Only muscular bruises (with muscle tissue affected) were classified according to their size in small (SB, when ≤ 8 cm of diameter), medium (MB, when > 8 and ≤ 16 cm of diameter), and large bruises (LB, when > 16 cm of diameter). The score of bruises severity per carcass (SBS, adapted from Anderson and Horder, 1979) was defined by multiplying the number of muscular bruises by the bruises' size classes weighting factors, which corresponded to weighing factor = 1 for small, weighing factor = 3 for medium, and weighing factor = 5 for large bruises, according to the following equation: SBS = number SB*1 + number MB*3 + number LB*5; and eight bruise points represent approximately 1 kg of bruised tissue trimmed per carcass.

2.6. Statistical analyses

All statistical analyses performed in the current study were carried out using the Statistical Analysis Software (SAS Institute Inc., Cary, NC, 2002) and a probability level of $p \leq 0.05$ was chosen as the limit for statistical significance in all tests.

Spearman's rank correlation was used to analyze the relationships between FS and RE over time. The values of FS and RE tests, at day 0 and final of feedlot period, were categorized into three classes namely (C = calm, I = intermediate and R = reactive). The FS classes (FSC) the definition was: calm (mean minus 0.5 STD), reactive (mean plus 0.5 STD) and the remainder were intermediate. For RE classes (REC) included calm (score 2 and 3), intermediate (4 e 5) and reactive (6 to 9).

The effects of temperament evaluated on day 0 (FSC_0 and REC_0) and on the final day in the feedlot (FSC_{fin} and REC_{fin}) on adrenal gland variables (ADCOR, ADMED, ADPER and ADWEIGHT) were analyzed by PROC MIXED. The model included space allowance (6, 12, and 24 m^2), breed (Nellore purebred and crossbred bulls), temperament classes (according $FSC_{0/fin}$, $REC_{0/fin}$, analyzed separately) and all interactions, as fixed factors. The space allowance by breed interaction was used as the error term for the analysis of space allowance and breed effects. Comparisons between the levels of temperament (calm, intermediate and reactive) were corrected by a Tukey adjustment.

The adrenal gland variables were also categorized into three classes ("low", "medium", "high"). For adrenal gland variables the definition was: "low" (mean minus 1.0 STD), "high" (mean plus 1.0 STD) and the remainder were "medium", for each space allowance. The effects of classes of adrenal ($ADCOR_C$, $ADMED_C$, $ADPER_C$, $ADWEIGHT_C$) on SBS were analyzed by PROC GLIMMIX. The models included space allowance (6, 12, and 24 m^2) and classes ("low", "medium" and "high" for $ADCOR_C$, $ADMED_C$, $ADPER_C$, $ADWEIGHT_C$) and their interaction as fixed factor. Breed was not included in the statistical model because it had not effect on adrenal gland variables. Comparisons between the classes of adrenal gland ("low", "medium" and "high") were corrected by a Tukey adjustment.

The descriptive statistics (mean, STD and CV) for the FS and RE at day 0 and at day final and morphometric variables of adrenal gland are shown in Table 2.

Table 2. Descriptive statistics for flight speed (FS) and reactivity (RE) tests at day 0 (FS_0, RE_0) and at day final (FS_{fin}, RE_{fin}) and all adrenal variables (ADCOR, ADMED, ADPER, ADWEIGHT)

Traits	Mean	STD	CV, %
FS_0, m/s	1.71	0.65	38.07
FS_{fin}, m/s	1.25	0.55	43.98
RE_0	3.79	1.10	29.05
RE_{fin}	3.73	0.73	19.69
ADCOR[3], cm^2	2.33	0.55	23.71
ADMED[4], cm^2	3.85	0.87	22.68
ADPER[5], cm	9.26	1.29	13.95
ADWEIGHT[6], g	8.63	1.33	15.42

[3]ADCOR: cortical area of adrenal glands per 100 kg of carcass
[4]ADMED: medullar area of adrenal glands per 100 kg of carcass
[5]ADPER: perimeter of adrenal glands per 100 kg of carcass
[6]ADWEIGHT: total adrenal weight per 100 kg of carcass

3. Results

3.1. Correlations between FS and RE over time

The correlation coefficient between FS values over feedlot time was significant and positive ($FS_{0,fin}$: r = 0.42, $p < 0.0001$, data not shown). But for RE measurements we did not observer significant correlation ($RE_{0,fin}$: r = 0.08, $p = 0.26$, data not shown), Both indicators were weakly significant correlated at the beginning of feedlot period (FS_0, RE_0: r = 0.26, $p < 0.0001$, data not shown), but not at the end of feedlot (FS_{fin}, RE_{fin}: r = 0.08, $p = 0.22$, data not shown).

3.2. Effect of bulls' temperament on adrenal gland morphometry

3.2.1. Using FSC_0 and REC_0

The FSC_0 had effect on ADPER and ADWEIGHT, with reactive bulls showing greater ADPER ($p = 0.01$) than the calm ones, and heavier ADWEIGHT ($p < 0.01$) than the calm and intermediate ones (Table 3). A significant interaction was also found between space allowance and FSC_0 on ADCOR for bulls kept at 6 m^2 (C = 2.41; I =

2.55; R = 2.73; SEM = 0.31; p = 0.02) and at 12 m^2 per animal (C = 2.66; I = 2.42; R = 3.25; SEM = 0.50, p < 0.01), reactive bulls presented a greater ADCOR than intermediate and calm ones. At 24 m^2 per animal no significant difference in ADCOR was found among temperaments (C = 2.07; I = 2.10; R = 2.12; SEM = 0.33, p = 0.92).

Significant interaction (p= 0.01, Table 3) between breed and FSC_0 influenced ADMED, with calm Nellore purebred bulls presenting smaller ADMED than intermediate and reactive ones (C = 2.68; I = 3.16; R = 3.33; SEM = 0.21; p = 0.01). Whereas, for crossbred bulls, calm and reactive had higher ADMED than the intermediate animals (C = 3.68; I = 3.29; R = 3.72; SEM = 0.21; p < 0.01).

Table 3. Least square means of adrenal gland measures for each class of temperament (C = calm, I = intermediate and R = reactive) based on assessments on day 0 in the feedlot according to flight speed (FSC_0) and reactivity (REC_0) classes [1,2]

					p-value				
	C	I	R	SEM	SA	Breed	FSC REC	SA* FSC/REC	breed* FSC/ REC
FSC_0									
ADCOR[3], cm^2	2.38	2.36	2.70	0.22	0.58	0.78	< 0.01	**0.04**	0.79
ADMED[4], cm^2	3.15	3.20	3.56	0.17	0.47	0.12	0.02	0.47	**< 0.01**
ADPER[5], cm	8.84^b	9.20^{ab}	9.72^a	0.29	0.59	0.53	**0.01**	0.26	0.33
ADWEIGHT[6], g	8.22^b	8.46^b	9.35^a	0.33	0.79	0.44	**< 0.01**	0.12	0.31
REC_0									
ADCOR	2.27	2.38	2.56	0.27	0.60	0.82	0.04	**< 0.01**	0.71
ADMED	3.14	3.25	3.25	0.38	0.72	0.33	0.96	0.95	0.87
ADPER	9.31	9.03	9.50	0.54	0.51	0.75	0.08	**0.01**	0.40
ADWEIGHT	8.93	8.49	8.78	0.69	0.75	0.76	0.30	**0.01**	0.69

[1]Within a row, means with a different letter differ (P < 0.05) by Tukey adjustment
[2]SA: space allowance (6, 12 or 24 m^2 per animal); Breed: Nellore purebred and crossbreed
[3]ADCOR: cortical area of adrenal glands per 100 kg of carcass
[4]ADMED: medullar area of adrenal glands per 100 kg of carcass
[5]ADPER: perimeter of adrenal glands per 100 kg of carcass
[6]ADWEIGHT: total adrenal weight per 100 kg of carcass

The main effects of space allowance, breed, and REC_0 were not significant, neither the interaction between breed and REC_0 (p > 0.05, Table 3) for all adrenal measures. Significant interactions between space allowance and REC_0 were found for ADCOR (p < 0.01), ADPER (p = 0.01) and ADWEIGHT (p = 0.01), and the respective adjusted means are presented in Table 4.

Table 4. Interactions between space allowance and reactivity classes on day 0 (REC_0) (C = calm, I = intermediate and R = reactive) on the adrenal gland measures [1,2]

	SA6				SA12				SA24			
REC_0	C	I	R	SEM	C	I	R	SEM	C	I	R	SEM
ADCOR[4], cm^2	2.37	2.59	2.58	0.33	2.50^b	2.39^b	3.07^a	0.51	1.95	2.16	2.02	0.40
ADPER[5], cm	9.01^b	8.90^b	9.72^a	0.54	10.19^a	8.96^b	10.03^a	0.74	8.71	9.24	8.75	0.80
ADWEIGHT[6], g	8.53^b	8.62^b	9.27^a	0.71	9.80^a	8.25^b	8.84^a	0.92	8.45	8.59	8.24	0.91

[1]Within a row, means with a different letter differ ($P < 0.05$) by Tukey adjustment
[2]SA6 (space allowance of 6 m^2 per animal), SA12 (space allowance of 12 m^2 per animal); SA24 (space allowance of 24 m^2 per animal);
[3]ADCOR: cortical area of adrenal glands per 100 kg of carcass
[4]ADPER: perimeter of adrenal glands per 100 kg of carcass
[5]ADWEIGHT: total adrenal weight per 100 kg of carcass

Reactive bulls kept at 6 m^2 per animal had greater ADPER and heavier ADWEIGHT when compared with calm and intermediate ones ($p < 0.05$). Whereas, the space allowance of 12 m^2 per animal produced a lower ADPER and ADWEIGHT in the intermediate bulls than the calm and reactive ones, and larger ADCOR in reactive bulls. No difference in adrenal gland morphometry variables was found as a function of bulls' temperament, for animals kept at 24 m^2 per animal ($p > 0.05$) (Table 4).

3.2.2. Using FSC_{fin} and REC_{fin}

FSC_{fin} only influenced ADPER, with the perimeter of the adrenal gland being larger ($p = 0.01$) in reactive bulls than in the intermediate and calm ones. For REC_{fin}, no main factor of space allowance, breed or REC_{fin} neither any interaction was found on all adrenal gland traits ($p > 0.05$) (Table 5).

Table 5. Least square means of adrenal gland variables for each class of temperament (C = calm, I = intermediate and R = reactive) based on assessment on final day in the feedlot, according to flight speed (FSC_{fin}) and reactivity (REC_{fin}) classes [1,2]

					p-value				
	C	I	R	SEM	SA	Breed	FSC REC	SA* FSC/REC	breed* FSC/REC
FSCfin									
ADCOR[3], cm^2	2.39	2.44	2.52	0.20	0.57	0.66	0.52	0.68	0.85
ADMED[4], cm^2	3.32	3.30	3.28	0.12	0.50	0.22	0.97	0.97	0.59
ADPER[5], cm	8.96^b	9.21^b	9.78^a	0.32	0.89	0.46	**0.01**	0.58	0.30
ADWEIGHT[6], g	8.31	8.63	8.98	0.39	0.89	0.43	0.10	0.99	0.91
RECfin									
ADCOR	2.54	2.44	2.48	0.41	0.61	0.85	0.92	0.91	0.88
ADMED	3.82	3.31	3.22	0.50	0.50	0.19	0.33	0.33	0.14
ADPER	9.48	9.18	9.18	0.94	0.82	0.92	0.92	0.67	0.78
ADWEIGHT	9.71	8.61	8.75	0.92	0.78	0.27	0.40	0.93	0.08

[1]Within a row, means with a different letter differ ($P < 0.05$) by Tukey adjustment
[2]SA: space allowance (6, 12 or 24 m^2 per animal); Breed: Nellore purebred and crossbreed
[3]ADCOR: cortical area of adrenal glands per 100 kg of carcass
[4]ADMED: medullar area of adrenal glands per 100 kg of carcass
[5]ADPER: perimeter of adrenal glands per 100 kg of carcass
[6]ADWEIGHT: total adrenal weight per 100 kg of carcass

3.3. Effect of adrenal gland morphometry on the severity of carcass bruises

Significant interactions between space allowance and ADMEDC ($p = 0.03$), ADPERC ($p < 0.01$) and ADWEIGHTC ($p < 0.001$) on SBS were found and are presented on Table 6. For "low" ADCORC, the SBS was zero, them analyses was performed for "medium" and "high" classes and were non-significant ($p > 0.05$) (Table 6). For bulls kept in 6 and 12 m^2 per animal, in "low" ADWEIGHTC, the adjusted means of SBS was zero, and then multiple comparisons in this variable were done between "medium" and "high" classes (Table 7). For bulls kept in 6 m^2 per animal, "high" ADMEDC, ADPERC and ADWEIGHTC, showed higher means of SBS compared to "low" and "medium" classes. For bulls kept in 12 m^2 per animal, "medium" ADPERC and ADWEIGHTC had significantly higher means of SBS than "low" and "high" classes. And for bulls kept in 24 m^2 per animal, "high" ADMEDC and ADPERC showed higher mean of SBS than "low". While that for ADWEIGHTC, the lowest mean of SBS was found for "medium", and "low" and "high" did not differ between them (Table 7).

Table 6. Adjusted means of score of bruise severity (SBS) for each class (CatAD = "low", "medium" and "high") of adrenal variables (ADCORc, ADMEDc, ADPERc, ADWEIGHTc) [1,2]

SBS	CatAD Low	CatAD Medium	CatAD High	SEM	SA	CatAD	SA*CatAD
ADCORc, cm^2	0.00	0.80	0.98	0.18	0.99	0.61	0.75
ADMEDc, cm^2	0.61	0.73	1.09	0.18	<.0001	0.06	**0.03**
ADPERc, cm	0.29	0.73	0.72	0.15	0.56	0.05	**<0.01**
ADWEIGHTc, g	0.00	0.70	1.16	0.21	0.99	0.03	**<0.001**

[1]CatAD: adrenal variables in classes (low, medium and high)
[2]SA: space allowance (6, 12 or 24 m^2 per animal); Breed: Nellore purebred and crossbreed
[3]ADCOR**C**: cortical area of adrenal glands per 100 kg of carcass, *in classes*
[4]ADMED**C**: medullar area of adrenal glands per 100 kg of carcass, *in classes*
[5]ADPER**C**: perimeter of adrenal glands per 100 kg of carcass, *in classes*
[6]ADWEIGHT**C**: total adrenal weight per 100 kg of carcass, *in classes*

Table 7. Interaction between space allowance and the classes (CatAD = "low", "medium" and "high") of adrenal variables (ADMED**c**, ADPER**c**, ADWEIGHT**c**) on score of bruise severity (SBS) [1,2]

SBS	SA6 Low	SA6 Medium	SA6 High	SEM	SA12 Low	SA12 Medium	SA12 High	SEM	SA24 Low	SA24 Medium	SA24 High	SEM
ADMEDc[4], cm^2	0.29[b]	0.44[b]	1.10[a]	0.33	2.00	1.37	1.18	0.53	0.39[b]	0.65[ab]	1.00[a]	0.28
ADPERc[5], cm	0.42[b]	0.32[b]	0.83[a]	0.19	0.40[b]	1.73[a]	0.44[b]	0.28	0.14[b]	0.69[a]	1.00[a]	0.28
ADWEIGHTc[6], g	0.00*	0.36[b]	1.82[a]	0.44	0.00*	1.61[a]	0.80[b]	0.28	0.64[ab]	0.58[b]	1.08[a]	0.31

[1]Within a row, means with a different letter differ ($P < 0.05$) by Tukey adjustment
[2]SA6 (space allowance of 6 m^2 per animal); SA12 space allowance of 12 m^2 per animal); SA24 (space allowance of 24 m^2 per animal)
[3]ADMED**C**: medullar area of adrenal glands per 100 kg of carcass, *in classes*
[4]ADPER**C**: perimeter of adrenal glands per 100 kg of carcass, *in classes*
[5]ADWEIGHT**C**: total adrenal weight per 100 kg of carcass, *in classes*

4. Discussion

In the present study, we tested the hypotheses that: *i*) the temperament has detrimental effect on adrenal morphometric variables; *ii*) enlarged adrenal glands would be related with the severity of bruises on carcass and; *iii*) these relationships would be more pronounced as the space allowances reduced.

The significant positive correlation between FS_0 and FS_{fin} indicated a moderate level of individual consistency in the expression of FS over time (Burrow and Dillon, 1999; Petherick et al., 2002; Muller and von Keyserlingk, 2005; Kadel et al., 2006). However, RE_0 and RE_{fin} was not correlated, in contrast with others results that reported significant correlation between measures (Cafe et al., 2011b, Turner et al., 2011). Likely we failed to find significant correlation due low sensitivity of test as reported by Curley et al., (2006) and Gibbons et al., (2011).

The FS_0 and RE_0 were positively correlated although the moderate value, but there was no significant correlation between FS_{fin} and RE_{fin}. Moderate value for the correlation between FS_0 and RE_0 and the absence of correlation between FS_{fin} and RE_{fin} suggest that, at beginning of feedlot, both measures were affected by overlapping underlying components, likely due novelty of environment and/or handling, rather than the same specific component (MacKay et al., 2013). After habituation process, that has been widely associated to feedlot period (Curley et al., 2006; Titto et al., 2010; Cafe et al., 2011b), FS_{fin} and RE_{fin} seem to address distinct aspects of temperament, supporting the arguments that FS and RE tests measure different combinations of the traits that contribute to cattle's temperament (Kilgour et al., 2006; Petherick et al., 2009; MacKay et al., 2013). However, what each test specifically measures remains unclear.

According to FSC_0, reactive bulls, independently of space allowance and breed had adrenal glands, on average, with 0.88 cm greater of perimeter (ADPER) and 1.13 g heavier (ADWEIGHT) compared to calm ones. Similar results were found at the end of feedlot where reactive animals according to FSC_{fin} had greater ADPER and a tendency to have heavier ADWEIGHT compared with calm ones.

The enlargement of adrenals glands has been reported as being highly correlated with the cortisol or corticosterone levels (Howes et al., 1960; Doornenbal, 1974; Carsia and Weber, 1986; Carsia et al., 1988), and changes in adrenals glands morphometry were reported to chronic stress states in different species (*sheep*: Panaretto and Ferguson, 1969 a,b; *monkey*: Shively and Kaplan 1984; *rats*: Alario et al., 1987; *broiler chickens*: Muller et al., 2015).

Our finding imply one step forward in the understanding that reactive bulls according to FSC_0 and, in parts, FSC_{fin} are in a possible state of chronic stress, as suggested by Curley Jr. et al. (2008) and Cafe et al. (2011a), that found a sustained higher cortisol basal concentration in reactive cattle compared to calm throughout the entire experimental period to CRH and/or ACTH pre-challenge. In this context, besides reactive temperament according to FSC_0, reduced space allowance is an important source of variation on ADCOR once the effect was not equally distributed among temperament classes. Clearly, reactive bulls kept on reduced space allowance (6 or 12 m^2/animal) had detrimental effect on ADCOR compared with calm and intermediate ones. In contrast, when kept on the largest space allowance (24 m^2/animal), no significant difference was found among temperament classes. Higher ADCOR occurs due the proliferation of adrenocortical cells, which result in cellular hypertrophy and hyperplasia in the fasciculate zone (Ulrich-Lai et al., 2006) by stimulation of stress-related hormones in the peripheral circulation (Harvey et al., 1984). These results suggest that the challenge of reduced space. as the worst environmental conditions (Mader, 2011; West, 2011; Macitelli, 2015) and the social tension (Kondo et al., 1989; Fell et al., 1999; Rodenburg and Koene, 2007), may have a more harmful effect on the reactive bulls. In fact, when the space allowance reduced from 24 to 6 m^2 per animal, ADCOR increased in 14.11%, 17.65% and 22.34% for calm, intermediate and reactive bulls, respectively.

Clearly, reactive bulls according FSC_0 in reduced space allowance had the highest biological costs in attempts to adapt to the environment, resulting in adverse effect on animal welfare (Broom, 1986; Veissier and Boissy, 2006). Additionally, in a non-restrictive nutritional environment, as feedlots, animals stressed with enlargement of adrenal gland may develop visceral obesity and the metabolic syndrome (Shively et al., 2009). Reduced performance of reactive bulls on feedlot could be related with this dynamics of energetic and metabolic costs (Petherick et al., 2002; Behrends et al., 2009; Cafe et al., 2011b).

Regarding ADMED, FSC_0 showed different results for Nellore and crossbred. Reactive and intermediate Nellore purebred bulls had greater medulla area than calm ones indicating that more temperamental animals may had hypertrophy of ADMED due to unleashed more “fight or flight” response (Selye, 1973), which includes activation of neurally-derived chromaffin cells in the adrenal medulla, which release catecholamines into the systemic circulation (Ulrich-Lai et al., 2006). However, for crossbred bulls, no difference was found between reactive and calm ones, suggesting that genetic composition could have different impact on fight or flight” response.

According REC_0, classes of temperament were not the main source of variation since the effect was not equally distributed among space allowances on ADCOR, ADPER and ADWEIGHT. Reactive bulls in the smallest space allowance (6 m^2/animal) had greater ADPER and heavier ADWEIGHT, in contrast with the largest space allowance (24 m^2/animal), where no difference was found on ADCOR, ADPER and ADWEIGHT among temperament classes. Unexpectedly, when the bulls were kept on 12 m^2/animal, reactive and calm bulls had similar ADCOR, ADPER and ADWEIGHT, suggesting that in intermediate space, both (calm and reactive animals) probably had similar strategies to cope with the environment. At the end of feedlot period, REC_{fin} did not show any relationship with morphometric variables of adrenal gland.

Regarding bruises, the “high” ADMED**c**, ADPER**c** and ADWEIGHT**c** in the smallest (6m^2/animal) and the largest (24 m^2/animal) space allowance had higher SBS while in intermediate space (12 m^2/animal), “medium” class showed higher SBS. These results reinforce the evidence that bulls with enlargement of adrenal gland and higher ADMED suggesting hypertrophy of medulla area, probably had a greater “fight or flight” response during pre-slaughter handling, increasing the chance of collisions against facilities, falls and others sources of accidents during handling, implying in the higher SBS. As widely accepted, adrenaline stimulates gluconeogenesis and lipolysis, which mobilize energy stores for vigorous ‘fight or flight’ activity (Cannon, 1914) and this may be one explanation for the high correlation between severity of bruising and elevate ultimate pH (Strappini et al., 2013, McNally and Warris, 1996) and some extension of chronic catecholamine has been reported in strenuous muscular activity (Tarrant, 1989).

5. Conclusions

We concluded that reduced space allowance in feedlots result in more detrimental effects on the welfare of reactive bulls than of the calm ones. Space allowance increased seems to be an important factor to minimize the chronic stress of reactive bulls in feedlots. Furthermore, it was clear that more stressed animals were more likely to have severe bruise on their carcasses.

Taken together, a step forward has been taken to support the link between reactive temperament of beef cattle and chronic stress state, what have theoretical and practical implications on animal welfare and on performance in feedlots environment. Further research, using indirect indicators of stress, as the morphology of pituitary gland and other organs as well the amount of fat in abdominal cavity, could contribute to a better understanding about the costs of keeping reactive animals in challenging environments, from animal welfare and performance perspectives.

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CAPÍTULO 4 - Considerações finais

Inquestionavelmente a adoção de confinamentos para a terminação de bovinos de corte impõe uma série de desafios aos animais, desde a mudança de dieta até severas restrições comportamentais. Experiências em confinamentos comerciais sugerem que uma proporção expressiva dos bovinos é incapaz de adaptar-se ao ambiente de confinamento, apresentando reduzido ganho de peso e maior ocorrência de doenças, sendo que não apresentam os mesmos problemas quando mantidos em pastagens. Resultados deste estudo são claros: bovinos que permaneceram ou se tornaram calmos ao longo do tempo de confinamento apresentaram desempenho superior e melhor qualidade das carcaças que aqueles que eram mais reativos, independentemente da composição genética e do espaço disponível nas baias de confinamento.

Adicionalmente, bovinos reativos apresentaram glândulas adrenais maiores e mais pesadas e que, quando mantidos em reduzidos espaços disponíveis, exibiram aumento da área cortical, sugerindo um possível estado de estresse crônico. De maneira clara, reduzido espaço disponível resultou em mais efeitos deletérios no bem-estar de bovinos reativos quando comparado com os calmos. E ainda, animais que possuíam glândulas adrenais com maior área medular, maior perímetro e peso, foram mais susceptíveis em apresentar hematomas graves nas carcaças.

Ressalta-se que o estudo apresentado nesta tese foi realizado em condições comerciais, adotando-se o tamanho dos grupos, a dieta e o regime alimentar, bem como os manejos de rotina típicos de confinamento comerciais ao ar livre nas condições tropicais brasileiras, o que torna nossos resultados adequados para discutir o impacto dessas condições de criação no bem-estar dos bovinos.

Nossos resultados sugerem ainda que a disponibilidade de espaço no ambiente de confinamento é um fator importante na adaptação ao ambiente, principalmente para os animais mais reativos. Diante disso, a implementação e a busca de estratégias que afetem positivamente a capacidade dos animais se adaptarem ao confinamento, se fazem extremamente importantes para o bem-estar animal bem como para a lucratividade do sistema. Adicionalmente, ganhos podem ser esperados na imagem do produto, uma vez que a sociedade de maneira geral considera reduzido o grau de bem-estar de animais confinados, devido à restrição de espaço, se comparado com o de animais mantidos em pastagem.

Projeções indicam números crescentes de bovinos terminados em confinamento no Brasil, e é muito provável que grande parte dos animais terá origem zebuína, que são reconhecidos por serem mais reativos. Assim, podemos considerar que os resultados do presente estudo mostram a relevância de nos preocuparmos com o efeito de reduzidos espaços disponível no confinamento para bovinos reativos. Gostaríamos de salientar que até o presente momento pouca atenção tem sido destinada a parcela de animais que enfrentam sérias dificuldades ou que são incapazes de adaptar-se ao sistema de confinamento, o que obviamente tem implicações econômicas diretas a esse sistema de produção.

O desenvolvimento de mais pesquisas que busquem avançar no conhecimento dos custos biológicos e econômicos advindos de manter bovinos reativos em ambientes desafiadores pode ser decisivo na formulação de estratégias de gestão aplicadas pelos confinamentos. Por exemplo, nesse estudo sugerimos que o aumento do espaço disponível por animal nas baias de confinamento para os animais reativos tem efeito positivo no bem-estar animal, promovendo avanços em direção a uma pecuária intensiva mais sustentável e ética. Ainda, acreditamos que a provisão de maior espaço disponível por animal é o primeiro passo em direção ao oferecimento de um ambiente de confinamento que oferece melhores condições para que os bovinos aumentem sua capacidade de adaptação.