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**Comportamento animal e atributos fisiológicos de vacas leiteiras
submetidas a ambientes com e sem sombreamento
durante a estação quente**

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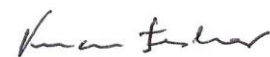
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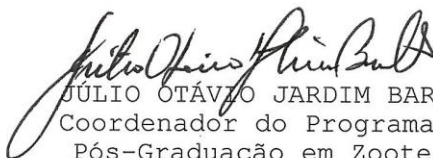
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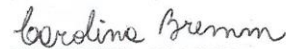
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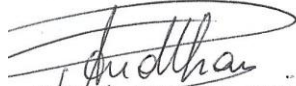
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RESUMO GERAL

¹Comportamento animal e atributos fisiológicos de vacas leiteiras submetidas a ambientes com e sem sombreamento durante a estação quente

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O ambiente físico tem grande importância na fisiologia do animal, influenciando a reprodução e produção. Com as mudanças climáticas e o melhoramento genético dos animais, com ênfase no aumento da produtividade por animal, os animais passaram a sofrer mais com as altas temperaturas, alterando seu comportamento e seu bem estar. O objetivo deste trabalho foi avaliar o comportamento social e ingestivo e atributos fisiológicos de vacas leiteiras durante a estação quente, com ou sem acesso à sombra. O estudo foi conduzido em Lages, SC, utilizando 14 vacas lactantes em pastejo, as quais foram divididas em dois grupos, permanecendo um grupo somente no sol sem acesso à sombra e o outro em ambiente com sombreamento. Foram observados os comportamentos ingestivo e social dos animais das 07:30 (GMT -2:00) às 23:00h (GMT-2:00), em intervalos de 10 minutos, totalizando 650min. As frequências respiratória e cardíaca, temperatura corporal das vacas e número de movimentos ruminais foram avaliados diariamente. Os dados do comportamento animal foram previamente padronizados e posteriormente, submetidos à análise multivariada, incluindo as análises de fatores principais de agrupamento, avaliação das variáveis que determinaram os agrupamentos por análise discriminante e canônica, utilizando o programa estatístico SAS 9.2. Os grupos diferiram principalmente quanto ao número de eventos de ingestão de água, competição por sombra e número de interações agressivas, aos tempos despendidos em ócio total, ócio em pé, de permanência perto do bebedouro, frequência respiratória e cardíaca, temperatura corporal, número de movimentos ruminais, escores de ofegação às 15 (GMT -2:00) e às 19 (GMT -2:00) horas e tolerância ao calor. No entanto os grupos de animais não diferiram quanto aos tempos gastos caminhando de cabeça alta, caminhando de cabeça baixa, em estação, número de eventos de competição por água no bebedor e os dados zootécnicos de dias em lactação, ordem de parto e produção de leiteira. O fornecimento de sombra na área de pastejo mesmo em condições moderadas de estresse térmico alterou positivamente os atributos fisiológicos e comportamentais. A severidade do estresse, percebida pela alteração dos atributos fisiológicos, alterou distintamente o comportamento social e ingestivo.

Palavras chaves: calor, comportamento social, comportamento ingestivo, conforto animal, frequência respiratória, frequência cardíaca.

¹ Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (66 p.) Março, 2014.

ABSTRACT

²Animal behavior and physiological attributes of dairy cows subjected to environments with and without shade during the warm season

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The physical environment, has a great importance on animal physiology affecting breeding and production. Due to climate change and animal breeding, which emphasizes higher production per animal, animals might get heat stressed from high temperatures, changing their behavior and their welfare. This study was performed to assess the social and feeding behavior and physiological attributes of dairy cows during the warm season with or without access to shade. The study was conducted in Lages, SC, using 14 lactating cows at grazing. These animals were divided into two groups, one group remained under the sunlight, without access to shade, while the other group was kept in shade-provided environment. Ingestive and social behavior of animals was observed from 7:30 (GMT -2:00) to 23:00 (GMT -2:00), registered at 10-minutes interval totaling 650 minutes. Respiratory and heart rate, body temperature and number of ruminal movements were assessed daily. The data of animal behavior were standardized using the standard procedure and then were subjected to multivariate analysis, including the analysis of key factors (PROC FACTOR), cluster analysis (PROC FATSCLUSTER AND PROC CLUSTER), evaluation of variables that determined groups by discriminant analysis and canonical, using SAS 9.2 statistical software. The groups differed mainly on the number of events of swallowing water, shade and competition for number of aggressive interactions, the time spent in complete idleness, loitering foot, staying near the water cooler, respiratory and heart rate, body temperature, number of ruminal movements, panting scores at 15 (GMT-2:00) and 9 (GMT-2:00) hours, heat tolerance. However animal groups did not differ regarding time spent walking head high, walking head down, season, number of events per water drinker and competition in the production data of days in milk, parity order and production of milk. The provision of shade in the paddocks, even under moderate heat stress, altered positively physiological and behavioral attributes. The stress severity, noticed by the change of physiological attributes, changed distinctly social and feeding behavior.

Key words: heat, social behavior, feeding behavior, animal comfort, respiratory rate, heart rate.

² Master of Science dissertation in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (66p.) March, 2014.

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LISTA DE ABREVIATURAS E SÍMBOLO

CN	near the water cooler
CWC	competition for water cooler
DW	walking head down
F	agressive interaction
FSH	hormônio folículo estimulante
GP	group
GT	total grazing
HGG	hipotalâmico-hipofisário-adrenal
HW	walking tall head
IW	ingest water
MR	ruminal movements
PM	milk production
RC	cardiac rate
RL	lying at rest
RR	respiratory rate
RT	total ruminated
RUL	lying ruminating
S	station
SC	competition for shade
SP 17	panting score at 17 hours
SP 21	panting score at 21 hours
SR	ruminating standing
SS	search for shade
ST	rest station
TH	heat tolerance
TL	lying total time
TR	rectal temperature
TRE	rest total

CAPÍTULO I

1. INTRODUÇÃO GERAL

O Brasil apresenta altas temperaturas do ar, em algumas regiões durante o ano inteiro e em outras somente no período de primavera e verão, as quais causam o sofrimento dos animais pelo calor, principalmente nos bovinos de leite de origem européia. Nacionalmente, cerca de 70% da produção de leite provêm de vacas mestiças Holandês-Zebu com predominância da raça Holandesa e existem poucas informações quanto à tolerância genética ao calor.

No sul do Brasil se concentra a maior predominância de rebanhos leiteiros da raça Holandesa, que evoluíram em regiões de clima mais ameno, e cujos mecanismos de dissipação de calor podem não ser suficientes para estes animais se adaptarem ao clima brasileiro. É nesta região que se encontram as maiores amplitudes térmicas, invernos frios e verões quentes. As temperaturas são influenciadas pela topografia, sendo mais frescas na região da serra. Entretanto, mesmo nestas localidades, durante os meses de verão (dezembro a março), os bovinos de leite têm sua produção alterada devido a altas temperaturas, superiores a 30°C, ultrapassando temperaturas de 21°C considerada por muitos autores como a zona de conforto térmico. Um ambiente é considerado termicamente confortável quando o animal está em equilíbrio térmico com o meio, ou seja, o calor produzido pelo metabolismo ruminal é perdido para o meio, sem prejuízo apreciável no seu rendimento. Quando esta perda de calor para o meio ambiente é aquém do seu incremento, ocorre estresse por calor.

O aumento das adversidades ambientais, sociais, patológicas e nutricionais provoca uma resposta endócrina clássica de estresse, liberando inicialmente adrenalina e noradrenalina e posteriormente glicocorticóides, acarretando modificações fisiológicas, bioquímicas e comportamentais nos animais. Estas alterações fisiológicas incluem alteração na temperatura corporal, aumento da transpiração, da frequência respiratória e conseqüentemente provocando o aumento da ingestão de água e ócio.

Os padrões de comportamento são reflexos da tentativa do animal de se libertar ou escapar de agentes ou estímulos estressantes. Essas reações podem ser usadas para identificar e avaliar o estresse e por oposição, o bem-estar. É através da alteração dos atributos fisiológicos como a frequência cardíaca, e do comportamento em geral, além da ocorrência de comportamentos anormais, que se pode observar a diminuição do bem estar. Todavia independente da região no Brasil, se observa pequena adoção de práticas que minimizam a carga térmica dos animais, o que implica em estresse leve a severo durante pelo menos 4 a 6 meses do ano. Na presente dissertação, foram avaliados como os animais reagem ao estresse térmico com ou sem acesso à sombra.

2. Revisão Bibliográfica

2.1 Fatores ambientais que influenciam o estresse térmico

O processo de criação de bovinos a pasto envolve uma série de fatores abióticos e bióticos que influenciam o comportamento dos animais, intervindo no seu desenvolvimento. Nesta relação: ambiente-animal, destacam-se os componentes abióticos relacionados ao clima. Temperatura, umidade, pluviometria e radiação solar são alguns dos fatores abióticos que influenciam a zona do conforto animal e também a taxa de crescimento das pastagens. Assim os estudos envolvendo aspectos climáticos são relevantes para a produção animal, especialmente em pastagem.

Segundo Hanh et al. (1999), West (2003) e Smith et al. (2013) o estresse se caracteriza pela soma de mecanismos de defesa do organismo em resposta a um estímulo provocado por um agente estressor externo e/ou interno, para manter o equilíbrio fisiológico, ocasionando respostas comportamentais, fisiológicas e imunológicas. Valores elevados de radiação solar (direta e indireta), de temperatura e umidade ambiental são os mais importantes fatores causadores de estresse para os animais criados a campo (Silanikove et al., 2000).

O ambiente térmico, principalmente em condições de campo, é bastante complexo, limitando sensivelmente a determinação da termorregulação, uma vez que a radiação, a velocidade do vento, a umidade e a temperatura do ar modificam-se no tempo e no espaço. Essas variáveis interagem entre si de modo que alteração de uma única variável ambiental pode alterar consideravelmente todos os fatores envolvidos no equilíbrio térmico dos animais (Silva, 2000).

Segundo Gonçalves et al. (2009), os bovinos, dependendo da raça e do nível de produção, possuem uma zona térmica considerada ótima para seu desempenho. Para as raças leiteiras, a zona de conforto representa uma variação da temperatura ambiente de 10 a 20°C, na qual a temperatura do corpo mantém-se constante, com o mínimo de esforço do sistema termorregulador. A amplitude maior da temperatura ambiente (5 a 25°C) é conhecida como zona termoneutra, os animais mantêm a homeotermia por meio de trocas de calor com o ambiente, lançando mão de mecanismos fisiológicos, comportamentais e metabólicos.

Existem alguns mecanismos de troca de calor (animal – ambiente): a condução, a convecção, a radiação e a evaporação. A condução é o processo no qual o animal troca calor com o meio pelo contato direto. A convecção é um processo de transporte de massa caracterizado pelo movimento de um fluido devido à sua diferença de densidade, especialmente por meio de calor, no animal se dá através da saída do calor do corpo para o ar mais frio, este processo é bastante influenciado pela velocidade do ar (ventos). A radiação é a ação direta dos raios solares sobre o animal, também se manifesta pela reflexão dos raios solares do solo e das instalações para o animal (Leme et al., 2005). A evaporação ocorre durante a respiração e transpiração pela pele e é bastante influenciada pela umidade do ar.

O resfriamento evaporativo é a principal forma de perda de calor disponível aos homeotérmicos quando a temperatura ambiente está maior que

a temperatura corpórea, e é mais eficiente quando a umidade relativa do ar é baixa (Robinson et al., 2004). Em ambiente de temperatura muito elevada, tanto o excesso como a carência de umidade serão prejudiciais. Se o ambiente é quente e muito seco a evaporação é rápida, podendo causar irritação cutânea e desidratação geral; no caso do ambiente ser quente e demasiadamente úmido, a evaporação torna-se muito lenta ou nula, reduzindo a termólise e aumentando a carga de calor do animal, principalmente porque, em condições de alta temperatura, a termólise por convecção é prejudicada (Starling et al., 2002). Por outro lado, a umidade elevada prejudica a termólise, aumentando o estresse pelo calor (Silva et al., 2000; Pereira, 2005). Embora a temperatura do ar seja frequentemente considerada como uma variável climática isolada de maior importância sobre a produção animal, seus efeitos estão intimamente ligados e dependentes do nível de umidade atmosférica (Silva et al., 2000).

As melhores condições de temperatura e umidade relativa para criar animais, em termos gerais, estão, respectivamente, em torno de 13 a 18°C e 60 a 70% de umidade relativa (Pires et al., 2004). Para gado de origem européia, os mesmos autores citaram que as condições adequadas se encontrariam em regiões com uma média mensal de temperatura abaixo de 20°C associada à umidade relativa em torno de 50 a 80%.

Nos últimos anos tem-se dado grande importância à temperatura de conforto das vacas lactantes, pelo fato que altas temperaturas, direta e indiretamente afetam negativamente a ingestão de alimentos, temperatura corporal das vacas, eficiência alimentar, produção de leite, eficiência reprodutiva e aumentam a incidência de doenças (Tucker et al., 2007; Rhoads et al., 2009, Wheelock et al., 2010). Os avanços obtidos na produção animal em áreas como nutrição e melhoramento genético resultaram em incremento expressivo da produção leiteira, o que resultou em maior taxa de calor metabólico e conseqüentemente aumentando a necessidade de dissipação de calor (Silanikove et al., 2000).

Com base em mudanças na produção de leite, se tem utilizado historicamente o índice de temperatura e umidade (ITU) de 72 como limiar superior de conforto térmico para o gado leiteiro. O ITU tem sido utilizado para descrever o conforto de animais, principalmente bovinos, desde que Johnson et al. (1962) relataram redução da produção de leite de vacas associada ao aumento do valor desse índice. Esse tem sido aplicado extensivamente em pesquisas no mundo todo, mesmo reconhecendo sua limitação, por não considerar os efeitos da velocidade do vento e radiação térmica. A temperatura do ar e a umidade têm grande influência nas trocas térmicas em ambientes quentes ou frios, e assim representam adequadamente o impacto nos animais (Hahn et al., 1997). Recentemente, um índice de carga de calor (HLI), que incorpora a radiação solar e velocidade eólica, tem sido usado como uma alternativa para o ITU (Gaughan et al., 2004).

A temperatura crítica superior para vacas lactantes da raça holandesa é 23°C e para o consumo de alimento a temperatura é entre 24 a 27°C. Campos et al. (2002) propuseram um valor máximo de 75 para ITU, visando ao conforto térmico do rebanho leiteiro da raça holandesa. Esses autores observaram que valores iguais ou inferiores a 70 não causaram

nenhum desconforto térmico para vacas leiteiras. Entretanto, para valores iguais ou superiores a 75, a produção de leite e ingestão de alimentos foram seriamente prejudicadas. Em recente revisão, foi sugerido que o valor mínimo de ITU para considerar a ocorrência do estresse térmico deve ser reduzido para 68 (Zimbelman et al., 2009).

2.2. Comportamento ingestivo de bovinos de leite em estresse térmico

De acordo com Baccari Júnior et al. (1998), devem-se considerar as seguintes implicações relacionadas à redução do consumo de alimento em animais sob estresse térmico: o hipotálamo controla diretamente a ingestão de alimentos e água; mudanças comportamentais, como procurar sombra, concorrem com a ingestão; maior ingestão de água inibe o apetite; a ofegação prejudica a ingestão; a redução da ingestão de alimentos está associada ao menor incremento calórico.

Segundo a terceira teoria do consumo voluntário “teoria termostática” (Curtis, 1983) com aumento da temperatura ambiental efetiva, o animal consome menos do que necessita para a sua produção, provavelmente devido à diminuição da atividade da tireóide, na tentativa de diminuir a produção de calor metabólico. À medida que a temperatura ambiente aumenta, ocorre a ativação do centro termorregulador sediado no hipotálamo, e este irá dar início a termólise, que vai fazer a evaporação respiratória aumentar através da ofegação. Se essa temperatura mantiver-se alta o animal irá diminuir o consumo de alimento, apresentará alterações comportamentais e com reflexos negativos sobre aspectos produtivos e reprodutivos (Baccari Junior et al., 1997).

Os reflexos negativos do estresse térmico se manifestam pela redução da produção leiteira (Paes Leme et al., 2005), alteração na produção dos componentes lácteos, como redução dos teores de proteína, gordura (Hammami et al., 2013), aumento dos teores de nitrogênio uréico, redução da estabilidade do leite (Abreu et al., 2011), contagem de células somáticas, redução do número de leucócitos e depressão do sistema imune (Fuquay et al., 2011).

Por outro lado, os efeitos negativos sobre a eficiência reprodutiva também ocorrem (Tao et al., 2013), estudos iniciais de Selye et al. (1936) mostraram que o estresse é acompanhado por um acréscimo na atividade do eixo hipotalâmico-hipofisário-adrenal e por um decréscimo na função reprodutiva, mostrando haver uma possível relação com os hormônios do eixo hipotalâmico-hipofisário-gonadal (HHG). Assim estes hormônios relacionados ao estresse podem influenciar a função sexual do eixo HHG: no hipotálamo, por meio do hormônio liberador de corticotrofina, onde este inibe a secreção de hormônio liberador de gonadotrofinas e, conseqüentemente, na hipófise anterior, diminui a liberação de hormônio luteinizante e de FSH hormônio foliculo estimulante, alterando nas gônadas o efeito estimulatório das gonadotrofinas e, assim, prejudicando a reprodução animal.

West et al, (2003) relataram que o impacto negativo das variáveis climáticas sobre a produção é defasada cerca de dois dias, possivelmente relacionada com alteração entre a ingestão e utilização de nutrientes consumidos, ou alterações no estado endócrino da vaca. As variáveis

ambientais estudadas durante o clima quente e a média do ITU, repercutiram nos resultados dois dias após realizado o registro. A produção de leite para vacas da raça Holandes declinou 0,88 kg por unidade de aumento no ITU, e para a média do ITU 72 (durante 2 dias consecutivos) o consumo de matéria seca declinou 0,85 kg para cada grau (°C) de aumento da temperatura média do ar a partir dos 24°C.

A severidade do estresse térmico parece ser dependente das flutuações diurnas e noturnas do ambiente. Se a temperatura ambiente assume valores abaixo de 21°C durante a noite por 3-6 horas, o animal tem oportunidade de dissipar o calor ganho durante o dia (Igono et al., 1992; Muller et al., 1994; West et al., 2003; Silanikove et al., 2009).

O estresse enfrentado pelos animais provoca alterações comportamentais, que envolvem mudanças posturais, tempo alocado às atividades, distribuição circadiana das atividades, consumo da dieta e de água, procura de sombra e atividades sociais. Os ruminantes são mais diurnos em seus hábitos ingestivos, sendo ativos durante o dia e repousando durante a noite. Entretanto, durante o clima quente em regiões tropical e subtropical, os animais ruminam e pastejam durante a noite, reduzem sua locomoção durante o dia e procuram sombra durante os dias quentes, isto é a consequência do comportamento de adaptação a estas áreas (Silanikove et al., 1987).

A redução do tempo de pastejo, especialmente diurno, é descrita por vários autores, como Kadzere et al.(2002); West, (2003); Silanikove et al. (2009). A redução no consumo também foi descrita por Silanikove et al. (2009), mas sua magnitude parece ser dependente de fatores como o teor de fibra das dietas, em função de sua ação sobre o incremento calórico (West et al., 1999); do nível de produtividade dos animais, resultando em um decréscimo de 17% na produção de leite de vacas de 15 kg de leite/dia e de 22% em vacas de 40 kg/dia (Porcionatto et al., 2009). Silanikove et al., (2009) relataram que temperaturas elevadas reduzem a frequência de alimentação nas horas mais quentes do dia, retardam o início do pico de pastejo à tarde e aumentam a frequência nas primeiras horas da manhã. O tempo de pastejo é normalmente de 8 horas durante o dia, isso ocorre porque a cada dia o animal distribui seu tempo entre as atividade de pastejo, ruminação e ócio, sendo que o pico de pastejo ocorre no início da manhã e no final da tarde quando a temperatura ambiente é menor (Januszkiewicz et al., 2011).

A ruminação é uma atividade comandada pelo sistema nervoso central e depende da quantidade de alimento presente no rúmen. Porém, é uma atividade que envolve grande produção de calor, e nas horas mais quentes do dia, os animais tendem a reduzi-la para, com isso, diminuir a produção de calor metabólico (Conceição, 2008).

Outro comportamento alterado é o de procura e ingestão de água. Segundo Peenington e Van Devender (2004), quando o ITU ultrapassa o valor de 80, o consumo de água aumenta em 50%, em parte para compensar as maiores perdas pela pele e respiração. A ingestão de água aumentou 1,2 kg para cada grau °C de aumento na temperatura ambiente mínima em estudo com 16 vacas multiparas holandesas observadas durante 5 meses com quatro diferentes dietas, pelo uso da regressão múltipla foi removida as diferenças

encontradas no consumo de alimento, para cada kg de leite produzido foi consumido 0,90 kg de água (Murphy et al., 1983).

2.3 Comportamento social e modificações posturais de bovinos de leite em estresse térmico

Entre as mudanças posturais associadas ao estresse térmico, se destacam o aumento do tempo em estação, o afastamento dos membros, o tempo em contato com superfícies mais frias (tempo deitado em superfícies frias e/ou molhadas). Essas modificações posturais foram associadas com o aumento de perdas de calor por convecção e por condução (Smith et al., 2012). No entanto, o aumento do tempo em estação (em pé) para 45% do dia pode aumentar diretamente a incidência de traumas nos membros e provocar o surgimento de novos casos de claudicação (Galindo & Broom, 2000; Privolo & Riva, 2009). Todavia o tempo deitado foi positivamente relacionado à produção de leite (Bach et al., 2008, Grant, 2007), estimando-se que, para cada hora de aumento do tempo deitado a produção de leite aumentou em 1,7 kg.

Dentre os padrões fixos de comportamento, o de deitar é considerado altamente prioritário para as vacas leiteiras que passam em média, entre 9 a 15 horas das 24 horas deitadas no *free stall*, mas isso depende do tipo de estábulo e do substrato da cama e do piso (Tucker et al., 2004).

Alen et al. (2013) combinaram três conjuntos de dados relativos a diferentes ensaios de estresse térmico realizados no Arizona (Anderson et al., 2012), Califórnia (S. Rungruang, inédito) e Minnesota (Smith et al., 2012). Em cada ensaio as vacas lactantes foram equipadas com dois registradores de dados: temperatura interna intra-vaginal e ângulo de perna para determinar o status deitado. No experimento de Minnesota, as temperaturas amenas resultaram em maior tempo deitado, enquanto nos dois outros experimentos, as condições meteorológicas vigentes mais severas foram relacionadas com maior tempo em estação. As vacas com temperatura corporal superior a 38,9°C permaneceram mais tempo em estação.

A disponibilidade de sombra pode modificar o comportamento dos animais e minimizar o efeito do estresse, pela redução da radiação direta e redução da temperatura ambiente (Baêta & Souza 1997; Conceição et al., 2008; Tucker et al., 2008). No entanto, fatores como o tipo de sombra, a área sombreada por animal, grau de interceptação da radiação solar influenciam a sua eficiência em minimizar o estresse calórico. O tempo de permanência na sombra aumentou com a capacidade de interceptação da radiação solar e a incidência ambiental de radiação solar. A capacidade de interceptação da radiação solar foi positivamente associada ao tempo em estação, mas não alterou o tempo deitado (Tucker et al., 2008).

A competição por recursos (alimento, água e áreas de sombra e repouso) pode ser agravada entre as vacas em pastejo com estresse térmico, tornando-se um importante fator de perturbação, gerando comportamentos agressivos e instabilidade social. Schutz et al. (2010) encontraram maior incidência (70%) de interações agressivas na menor área de sombra por vaca (2,4 m²/vaca) em comparação com a área de sombra mais abundante (9,6 m²/vaca). Hötzel et al. (2000), observando as interações comportamentais entre animais com redução na quantidade de água oferecida, identificou que vacas

dominantes passavam mais tempo perto do bebedouro após beberem água, aparentemente impedindo o acesso das vacas subordinadas ao recurso.

Segundo Phillips et al. (2002), a manutenção do espaço individual é um dos principais símbolos de *status* para os bovinos. O padrão de relações individuais observáveis numa população em uma determinada época pode ser definido como organização social. Essa não é uma característica fixa, típica da espécie, mas sim um padrão flexível de relações individuais que resulta da interação entre comportamento social, o ambiente e a história da espécie (Deag, 1981).

2.4 Respostas fisiológicas do estresse térmico: frequência respiratória e temperatura corporal

Existem diversos indicativos para caracterização do conforto e do bem-estar animal. Entre eles, está a observação criteriosa das respostas fisiológicas. West, (2003) cita que apesar do ITU ser uma ferramenta muito útil para medir a resposta da vaca ao estresse térmico, respostas individuais dos animais ao calor não devem ser desconsideradas. O primeiro sinal de estresse térmico evidenciado é o aumento na frequência respiratória (FR) e na eliminação de suor. O aumento na temperatura corporal (TC) é o resultado da falha na regulação da temperatura. Uma vaca leiteira em condições ideais de ITU apresenta TC entre 38 e 39,1°C e FR entre 15 e 35 movimentos por minuto. Uma TC > 39,2°C e FR > 60 movimentos por minuto é indicativo claro de estresse térmico (West, 2003).

O primeiro mecanismo acionado para perda de calor é a vasodilatação, o segundo é a sudorese e o terceiro é a respiração, sendo o aumento na FR o primeiro sinal visível. O aumento ou a diminuição da FR depende da intensidade e duração do estresse a que os animais estão submetidos (Martello, 2006). No entanto, este mecanismo de calor demanda energia, resultando no aumento de manutenção diária de bovinos de leite de 7 para 25%, o que também resultará em produção de calor (Columbiano et al., 2007).

Quando os mecanismos de termólise dos animais homeotérmicos não são eficientes, o calor metabólico somado com o calor recebido do ambiente torna-se maior que a quantidade de calor dissipada para o ambiente. Em consequência a isso, pode ser notado nesses animais um aumento da temperatura corporal. Com a temperatura corpórea elevada, o organismo reage aumentando a sudorese e a frequência respiratória para eliminar o excesso de calor (Morais et al., 2008).

O aumento da frequência respiratória por períodos longos, segundo Matarazzo et al. (2003), causa prejuízos ao organismo animal, tais como: redução no consumo de forragens, produção de calor endógeno adicional devido ao exercício da ofegação, desvio de energia para outros processos metabólicos e redução de CO₂ (acarretando em alcalose respiratória pelos baixos níveis de ácido carbônico no sangue). Com o objetivo de compensar a alcalose respiratória, o organismo aumenta a excreção de bicarbonato, pois os rins, na tentativa de controlar o equilíbrio ácido-base do sangue, aumentam a reabsorção de H⁺, HCO₃⁻ e cátions, primariamente o sódio (Na⁺), para serem excretados na urina ocasionando uma acidose metabólica moderada. Kadzere

et al. (2002) demonstram que para manter de forma efetiva o tamponamento sanguíneo, o corpo necessita manter uma relação de 20:1 de HCO₃- (Bicarbonato):CO₂ (Dióxido de Carbono).

Em condições normais de temperatura a vaca holandesa apresenta frequência respiratória em torno de 18 a 28 movimentos por minuto, a partir de 26°C de temperatura ambiente os movimentos começam a aumentar. Em temperatura de 31°C, vacas holandesas apresentam em média 68 movimentos por minuto. Até 60 movimentos, os animais não apresentam ainda sinais de estresse. Ultrapassando 28°C de temperatura ambiente e 120 movimentos, já refletem carga excessiva de calor e acima de 160 faz-se necessário adotar medidas emergenciais (Hahn et al., citado por Baccari et al., 2001). Para Silanikove et al. (2000), valores de FR de 40 a 60, 60 a 80 e 80 a 120 mov/min caracterizam respectivamente um baixo, médio e alto estresse para ruminantes, e acima de 200 mov/min o estresse é classificado como severo.

O limite de variação normal da temperatura retal (TR) de bovinos adultos é de 38,0 a 39,3°C, segundo Dukes et al. (1996). Dikmen & Hansen (2009) utilizaram dados ambientais e de TR oriundos de três fazendas leiteiras nos EUA (1.280 vacas em lactação), mantidas em *free-stall* climatizado (ventilação e nebulização). Os autores encontraram como valor crítico superior a temperatura de bulbo seco de 31,4 °C, associada com temperatura retal de 39,5 °C.

Wheeloch et al. (2010), estudando vacas em lactação em estresse térmico, sob temperaturas de 29,4 a 38,9 °C, alimentadas *ad libitum*, constataram que a temperatura retal e taxa de respiração, aumentaram durante o estresse térmico (38,6 - 40,4°C e 44-89 respirações / min, respectivamente). Brown- Brandl et al., (2005) recomendam que estudos futuros incluam a taxa de respiração como uma variável dependente, pois é um indicador mais sensível do estresse térmico do que a temperatura do corpo.

3. Hipótese e Objetivo

Hipótese

- O comportamento animal e as respostas fisiológicas são alterados favoravelmente pelo provimento de sombra, mesmo em condições de clima subtropical de altitude.

Objetivo geral

- Avaliar o comportamento social e ingestivo e variáveis fisiológicas de vacas em lactação durante a estação quente com ou sem acesso à sombra.

Objetivos específicos

- Avaliar atividades relacionadas ao comportamento social e ingestivo de vacas em lactação durante a estação quente com e sem acesso à sombra;
- Avaliar a temperatura corporal e as frequências cardio-respiratórias de vacas em lactação durante a estação quente com e sem acesso a sombra;

CAPÍTULO II
COMPORTAMENTO E ATRIBUTOS FISIOLÓGICOS DE VACAS
LEITEIRAS SUBMETIDAS A AMBIENTES COM E SEM
SOMBREAMENTO DURANTE A ESTAÇÃO QUENTE³

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Behavioral and physiological attributes of dairy cows subjected to environments with or without shading in hot season

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Implications:

Concern about animal welfare has become increasing in modern society, especially after the evidence that animals are sentient beings. Regardless of the controversies in the literature and scientific or technological backgrounds on global warming, high temperatures cause behavioral and physiological changes, reflecting the reduction of animal welfare. Consequently heat stress causes productivity losses, like the decrease in milk production and fertility as higher incidence of diseases and metabolic problems that together contribute to decrease the profitability of the production system. However, the adoption of measures by farmers to minimize the heat load on animals does not occur generally. In this sense, the provision of shade can mitigate the problems caused by solar radiation, helping to keep the behavioral and physiological patterns, even in subtropical environment of altitude, with milder temperatures.

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Abstract

The understanding of daily and seasonal climatic conditions, and the physiological and behavioral responses, allows the adoption of adjustments that promote great comfort to animals. This work aimed to evaluate the social and ingestive behaviors, besides physiological attributes of dairy cows in subtropical environment during the warm season with or without access to shade. The study was leading in two environments: with shade and without shade, for five days, using 14 lactating grazing cows, 10 Holstein and four crossbreds Holstein and Jersey. The collected data of animal behavior were standardized using PROC STANDARD and then were subjected to multivariate analysis, including the analysis of principal factors (PROC FACTOR), clustering (PROC CLUSTER and PROC FATSCLUSTER) and evaluation of the more determinant variables in the formation of clusters with discriminant and canonical analyzes, using SAS (9.2) statistical program. The group with no access to shade was positively associated with higher values of the measured scores panting at 15h (GMT - 02:00) and at 19h (GMT -02:00), Benezra's Animal Comfort Index, respiratory rate and aggressive interactions. Moreover, this group was negatively associated to the times spent seeking for shade and in rumination. They also presented moderate and negative correlation with milk production and the number of ruminal movements. The observations were pooled into three groups, and the most important variables in the classification of observations into those groups were panting score of the 19:00 (GMT -2:00) hours, the time spent grazing and water ingestion bouts. The groups differed mainly on the number of events of water intgestion bouts, competition for shade, aggressive

interactions, the time spent in complete rest, time spent in rest while standing, time spent around water trough, respiratory and heart rate, body temperature, number of ruminal movements, panting scores at 15 (GMT -2:00) and 19 (GMT -2:00) hours and Benezra's Animal Comfort Index. However clusters did not differ regarding time spent walking with head high, walking with head down, standing time, number of events of competition for water and for days in milk, parity order and production of milk. The animals with access to shade had the effects of heat stress minimized.

Key words: heat, social behavior, feeding behavior, animal comfort.

Introduction

The increase in the consumption of milk and the world's concern about animal welfare raise concerns of researchers and farmers on how to improve milk production and minimize interference that may cause discomfort to these animals (Lambertz et al., 2014).

Climate variables act directly on the animal, which seeks constantly to adapt to environmental conditions in the pursuit of welfare. Cattle in tropical and subtropical climates, especially those raised in pastures, are exposed to the sun and other weather elements for several hours a day. The intensification of livestock activity, reducing the space per animal and modification of the environment, often hinders the search for animals to find alternatives to adapt to these adverse conditions, making them more susceptible to heat stress and result in physiological changes that compromise productive performance (Deitenbach et al., 2008).

Moreover, advances made in animal nutrition and breeding increased expressively milk production per cow, which resulted in higher metabolic heat production, and therefore increased the need for heat dissipation (Silanikove et al., 2000; Baumgard et al., 2007).

In the subtropical area, places with high altitudes and humidity, the wind speed contributes to the heat dissipation of animals by convective processes, but it might be counteract by the high levels of humidity which reduces the dissipation of body heat by evaporative means (Azevedo et al., 2009). Evaporative cooling is the main way for heat loss when ambient temperature is higher than the body temperature, and this process is most efficient when the relative humidity is low (Robinson, 2004).

In the other side, provision of shade serves to mitigate the effect of solar radiation, reducing body temperature (Mitlohner et al., 2001 Kendall et al., 2006, Tucker et al., 2007), and thus can reduce the damage caused by high relative humidity air and contribute to evaporative cooling.

The hypothesis of this study is that animal behavior and physiological responses are altered favorably by providing shade even in the subtropical climate of altitude. The objective of this study was to analyze animal behavior and physiological attributes of lactating dairy cows with or without access to shade during the hot season.

Material and Methods

This study was approved by the Ethics Committee on Animal Use of Federal University of Rio Grande do Sul, project number 21901, within major

project "Etiology, characterization, mechanisms of action of nutritional and non-nutritional factors and prevention of milk unstable non-acidic (LINA)."

The experiment was conducted on days 28 (pre trial), 29, 30, 31 January and 1st February 2013, the dairy Farm State University of Santa Catarina (UDESC-CAV), located in the city of Lages - SC Brazil (latitude -27 ° 48 '58", longitude 50 ° 19' 34" and altitude of 950 meters above sea level), with a mean relative humidity of 79.3%, characterized by subtropical temperate climate (Cfb).

Fourteen lactating cows were used at grazing, 10 Holstein and four crossbred Holstein x Jersey. Cows were selected from the experimental herd to constitute a similar group in relation to milk production, order of birth, breed, lactation period and SCC. Later this group was split into two groups with seven cows each, which were placed in two paddocks (A and B) with 0.8 ha each, composed by Sudão grass (*Sorghum sudanense* L.) and Papuã grass (*Brachiaria plantaginea*). One group had access to shade, while the other group did not.

Cows were milked twice daily at at 9:00 GMT-2: 00 and 20:00 GMT-2: 00 and supplementation was provided before the two milkings. This supplement besides the pasture on offer provided the nutritional requirements for 25 kg of milk yield (NRC, 2001) and was composed of 200 g/kg soybean meal, 750 g/kg ground corn, 30g/kg mineral salt *new bovigold of Tortuga* and 20 g/kg sodium bicarbonate. The supplement contained 88% DM, 16.% CP, 8% NDF and 75 % TDN/kg (DM basis).

At the beginning of the trial, the animals showed 537.6 ± 93.5 kg of body weight, body condition score 2.8 ± 0.3 , 2.7 ± 1.5 lactations, 20.6 ± 6.5 kg milk/day and 149.3 ± 48.9 days in milk. During the adaptation phase and the first experimental day, shade was available to all cows, and during the next four days, shade was available just to one group of the cows.

Cows were observed for five days, from 07:30 (GMT-02:00) to 23:00 (GMT-02:00) from sunrise to sunset, totaling 960 minutes. The animals in different treatments had visual and auditory contact with each other. The volume of water consumed was measured daily in each paddock, with water meters. The results of the water intake are presented descriptively. Behavior was not recorded during periods of displacement of animals to and from the milking parlor, during the milkings and during the supplement feeding totaling an average of 350 minutes a day. Animals were visually observed by previously trained people, and the following activities of feeding behavior repertoire were recorded at 10-minute intervals: time spent ruminating, ruminating while lying, ruminating while standing, resting (no jaw movements), resting while lying, resting while standing, total standing time (standing ruminating or resting), total lying time (lying resting or ruminating), walking head down, walking head high, grazing.

The following activities were observed continuously: number of water ingestion bouts, permanence around the water trough, seek for shade (the animals moved to find shade or remained in the shadow made by another cow), competition for shade (attempts to displace other animal from the shade), competition for water trough (attempts to displace other animal from the trough)

and aggressive interactions (actions of intimidation or confrontation with other animals, with no competition for shade or water trough).

Data as days in milk, body weight, milk production and body condition score were evaluated in the first and last day of the experimental period. Cows were individually weighed before the morning milking. The body condition score was assigned to animals (BCS: 1-5 scale; Edmonson et al., 1989). Milk production was measured using milk meters on each milking during the five days.

Pasture samples were hand plucked in five locations in the paddocks before the beginning of the trial (Table IV). Pasture was cut within the area of a square of 20 x 20 cm. The contents of dry matter, crude protein, crude fiber, ether extract and ash were determined (AOAC, 2004). Pasture of paddock with access to shade contained 170.5 g/kg DM of crude protein, 208.2 g/kg of crude fiber and 691 g/kg of TDN while pasture of the paddock without shade contained 155.7 g/kg DM of crude protein, 224.3 g/kg Mof crude fiber and 711.5 g/kg of TDN.

The measurement of air temperature and relative humidity was performed by portable data logger model HT-500 weather station placed at 1,5 m above the ground and installed in the shaded area of paddock A and at the sunny area of the paddock B.

The temperature and humidity index (THI) was used as an indicator of thermal comfort and was calculated using the air temperature and humidity measured at 11 (GMT-2: 00) and at 17 (GMT-2:00) for all experimental days using the formula (Johnson et al, 1962):

$$THI = (1,8X Tdb + 32)-[(0.55-0.0055 x RH) X (1.8 X Tdb - 26.8)],$$

where Tdb = dry bulb temperature in ° C and RH = relative humidity, %.

The respiratory and heart rates, body temperature of cows and number of ruminal movements were assessed daily before the animals were milked in the afternoon.

Body temperature (RT) was measured using a clinical veterinary thermometer inserted near the rectum of the animal wall at a depth of approximately 5 cm. Heart rate (HR), expressed in number of beats per minute, was measured using a stethoscope and stopwatch for 30 seconds and multiplying the result by two to obtain this variable in minutes. The respiratory rate (RR), expressed in number of breaths per minute, was measured using a stethoscope and stopwatch upon auscultation of respiratory movements for 30 seconds and the value obtained multiplied by two to obtain this variable in minutes.

The panting score (Mader et al, 2006) was assigned by an observer in the field at 11 daily (GMT-2: 00) and 17 (GMT-2: 00) and ranked zero = no panting to four = severe panting. The Benezra's Animal Comfort Index (HT) was calculated using the equation: $HT = (TR / 38.33) + (FR / 23.00)$.

Statistical Analysis

Cows were considered experimental units and they were randomly assigned to two treatments (area with access to shade and area without access to shade). Data was previously standardized using PROC STANDARD (mean zero and standard deviation = 1), and subsequently submitted to multivariate

analysis, including the analysis of principal factors (PROC FACTOR), cluster (PROC FASCLUS and PROC CLUSTER) and evaluation of the variables that determined groups by discriminant and canonical analyzes (PROC STEPDISC) (PROC CANDISC) using SAS (9.2) statistical program. Attribute's values of the clusters were subjected to multivariate analysis of variance with PROC GLM, MANOVA option. Means were tested using Tukey test with a significance level of 5%.

Results

There was great variation in temperature and relative humidity between morning and afternoon and between areas with and without shade (Table I), which influenced the results of THI. In general, the values of temperature and THI were lower at 11 h (GMT-2: 00) compared with the values obtained at 17 h (GMT-2:00), and lower in shaded area than in the unshaded one. Regarding the relative humidity, the lowest values generally occurred at 17 h (GMT-2:00) and in the paddocks without shade. The highest values of water consumption occurred in the first day for the group of cows without access to shade, 72.9 l compared to the cows with access to shade, 58.6 l. In the others days,

Seven principal factors had eigenvalues greater than one, and the first two of them explained, respectively, 27.9% and 14.4% of the total variance. In multivariate analysis, considerations can be performed by analyzing the angle between the vectors of the main factors. Angles of 0 to 180° between the variables of the plane between factors 1 and 2 indicate correlation, respectively,

positive and negative, while angles of 90 ° between the variables indicate low or no correlation between them (Smith et al. 2002). Furthermore, the distribution of variables in the plane orthogonal to the right and left of the vertical axis and above and below the horizontal axis indicate respectively positive and negative associations between features.

The group of animals without access to shade was positively associated with higher values of the scores panting measured at 15h (GMT - 02: 00) and at 19h (GMT - 02:00), Benezra's Animal Comfort Index, respiratory frequency, body temperature, heart rate and number of agonistic interactions, the events of water ingestion bouts, permanence around the water trough, competition for shade and the total grazing time (Figure 1). This same group of animals was negatively associated with the time of seeking for shade and the time spent in rumination, milk production and the number of ruminal movements (Figure 1).

Secondly, considering the distribution of the variables in the orthogonal plane, above and below the horizontal axis, it was found that some animals in the group without access to shade were positively related to the time spent ruminating while standing, total standing and resting while standing, but negatively associated with the total time spent lying, lying while ruminating and lying while resting.

The discriminant analysis shows that 12 attributes were used to distinguish the groups ($P < 0.10$), whereas the score panting at 19:00 (GMT - 2:00), grazing time, water intake bouts, number of aggressive interactions,

respiratory rate and time spent lying were the most significant variable (Table II).

The canonical analysis of variables shows that observations were gathered in three groups: group one with 27 observations, group two with 34 observations and group three with nine observations (Figure 2). Both canonical correlations were significant ($P < 0.0001$). Group one was most closely associated with times spent lying, grazing and seeking for shade and number of ruminal movements. Group two was associated with rectal temperature, time spent resting, resting while standing, number of competition for shade events, water intake bouts. In the other hand group three was mostly related to times spent standing, ruminating while standing and number of competition events near the water trough (Figure 2).

Group one showed higher values for times spent lying, lying while resting and seeking for shade, number of ruminal movements compared with groups three and two. Group two presented higher values for the time spent grazing. Group three showed higher values for times spent on resting, resting while standing, number of competition events for shade and number of competition events near the water trough, number of water intake bouts, number of aggressive interactions, panting score at 15h (GMT - 02 : 00) and 19h (GMT - 02: 00) and rectal temperature when compared with groups one and two. Groups one and two had the highest averages for total time spent ruminating and time spent ruminating while lying. In the other hand groups two and three had the highest mean for heart rate, respiratory rate and HT. Groups

did not differ regarding time spent walking head high, walking head down, days in milk, parity order and production of milk (Table III).

Discussion

Overall, the observations were grouped into the three clusters by physiological and adaptive postural changes to deal with heat stress and lack of shade. The region of Lages, SC presents huge weather variations between diurnal and nocturnal phases of the day (Antunes et al., 2004). Variations of THI, air temperature and relative humidity values observed in this study support this assertion (Table II).

One may expect potential and beneficial effects of natural cooling during the nocturnal phase over behavioral and physiological attributes during the diurnal phase. Indeed compensations over milk production have been reported (Silanikove et al., 2009). However from the welfare perspective is not clear whether cows may anticipate this relief during the night and change their behavior.

Although cow's behavior was not evaluated during the night and the trial was very short to perceive changes in BCS and BW, we might infer by the very similar milk yield (Table III) and composition (data not shown) that cows compensate at least time spent grazing. However, despite this possible compensation during night time, providing shade even in high altitude improves daytime thermal comfort, decreasing both minimal and maximal THI values as in the absence of shade, THI ranged from 75.5 to 79.6, whereas in shaded environment THI ranged between 72.7 and 78.6.

All cows were heat stressed if we consider that their physiological attributes as their respiratory rate and body temperature were above values reported to heat unstressed animals. But there were differences between animals for behavior and physiological attributes which could not be entirely related to the provision of shade, as observations were grouped in three groups and the number of them related to more stressed animals overcame the number of cows*days of animals deprived of shade.

Comparison between groups showed that, in group one (Table III) heat stress may be considered as mild, since, although the average body temperature was 39.6°C, respiratory rate was 57.33 and the panting score was slightly greater than zero (Collier et al., 2012). In the other hand, in group two heat stress may be considered as moderate while in group 3 heat stress might be considered as severe, as both groups exhibited higher values for respiratory rate, panting scores and rectal temperature, but in group three animals showed the highest values.

From now on, groups one, two and three will be denominated as mild, moderate and severe stress, respectively. When the heat dissipation mechanisms become insufficient, rectal temperature tends to increase (Morais et al., 2008) and consequently the animals without access to shade had higher rectal temperatures, as observed in the SE group. The increases in panting score values and respiratory rates were expected since at temperatures above the minimum critical temperature heat loss via evaporation progressively loses importance and losses due to peripheral vascular dilation and evapotranspiration become increasingly more important (Kadzere et al., 2002).

In the present study, the positive relationship between panting score in the group of animals without access to shade and increased respiratory rate, seen in figures one and two, occurred due to the exposure to the sun these animals were submitted, storing heat. The animals without access to shade had larger water intake and greater number of water ingestion bouts, what is in agreement with previous studies (Murphy et al., 1983; Schütz et al., 2014) and is related to the increased body fluid loss caused by the augmented sweating, heart rate and salivation (Atrian et al, 2012).

The highest values of resting time in the severely stressed group may be related to the strategy of animals to reduce physical activities (West, 2003) that increase heat production as grazing and especially ruminating, and also they may be related to the increment in the activities related to water intake and permanence around the water trough. The increase in time resting while standing is probably related to postural changes adopted to increase heat dissipation by convection, which was also noticed by Anderson et al. (2013) and Rungruang (in press). The increase of the competition for shade which is motivated by the need to decrease the high body temperature, may involve frustration and anxiety, as the number of aggressive interactions increased in the groups intensively heat stressed, as already noticed by Tresoldi (2012). In the present study cows without access to shade could see the other cows using the shade of trees. However the similar number of events of competition near the water trough indicates that although the use of the water trough increased for more heat stressed cows, the access was not limited, and thus it did not elicit more agonistic events.

As expected the act of seeking for shade prevailed in the group of cows that had access to shade, serving as an alternative to keep the animal in the comfort zone, and reducing body temperature.

Milk production was not significantly different between groups, possibly because they were not so challenged due to the low milk production of all animals before the trial (Baccari Jr et al., 1982; West, 2003; Vasconcelos and Demetrio, 2011; Collier et al., 2012), and due to the similar parity and DIM, besides the possible compensation in DM intake during the night (Kadzere et al., 2002; Silanikove et al., 2009) whereas animals with higher yield potential are more sensitive to heat stress. During the night-time average air temperatures were 18.84 °C. The severity of heat stress depends on the diurnal and nocturnal fluctuations of the weather. If the ambient temperature has values below 21 °C overnight for three-six hours, the animal has a chance to dissipate heat gain during the day (Igono et al., 1992; Muller et al., 1994; West, 2003; Silanikove et al., 2009), which may allow similar DM intake and milk production, especially if the animals are not very productive.

In general, as severity of heat stress augmented cows exhibited increasingly more changes in physiological and behavioral attributes notably those more related to dissipation of heat but also those related to anxiety and frustration. Cows may compete for resources that can mitigate heat stress, such as water and shade, to the point where the benefits outweigh the costs. On the other hand, when this ratio is to be reversed, that is, when the costs outweigh the benefits, the animals are able to change their strategies, preventing agonistic interactions, saving energy and preventing excessive heat

production. However animals may have different strategies when face the same challenges which can also be observed in the present study.

Conclusions

Access to shade, even in moderate conditions of heat stress, positively affected the physiological and behavioral attributes of cows at pasture. The severity of stress perceived by the change of physiological attributes distinctly altered the social and ingestive behavior of animals.

References

- Anderson SD, Bradford BJ, Harner JP, Tucker CB, Choi CY, Allen JD, Hall LW, Rungruang S, Collier RJ and Smith JF. 2013. Effects of adjustable and stationary fans with misters on core body temperature and lying behavior of lactating dairy cows in a semiarid climate. *Journal of Dairy Science* 7, 4738-4750.
- Atrian P and Shahryar HA. 2012. Heat stress in dairy cows (a review). *Res. in Zoology* 2, 31-37.
- Antunes LEC and Rasseira MCB. 2004. Aspectos técnicos da cultura da amora preta. Documentos, 122, Embrapa Clima Temperado, BR.
- Association of Official Analytical Chemists 2004. Official methods of analysis, 2 vol., 18th edition. AOAC, Arlington, VA, USA
- Azevêdo DMR and Alves AA. 2009. Bioclimatologia aplicada à produção de bovinos leiteiros nos trópicos. Embrapa Meio-Norte, Teresina, BR.
- Baccari JR F, Assis PS, Polastre R. et al. 1982. Shade management in tropical environment for milk production in crossbred cows. *Proceedings Western Section American Society of Agricultural Engineering* 33, 209-210.
- Baumgard LH and Rhoads RP. 2007 The effects of hyperthermia on nutrient partitioning. *Proc. Cornell nutr. Conf...* Arizona, EUA.
- Collier RJ and Collier JL. 2012. *Environmental Physiology of Livestock* (Eds, Jonh Wiley & Sons) pp 368 Oxford, Garsington Road,
- Deitenbach A, Floriani GS and Dubois JCL et al. 2008. Manual agroflorestal para a Mata Atlântica, 1ª edition. Ministério do Desenvolvimento Agrário, Brasília, BR.
- Edmonson AJ, Lean IJ, Weaver LD, Farver T and Webster G. 1989. A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* 72, 68-78.

- Igono MO, Bjotvet G and Sanford-Crane HT. 1992. Environmental profile and critical temperature effects on milk production of Holstein cows in desert climate. *Int. J. Biometeorol* 20. 36, 77–87.
- Kadzere CT, Murphy MR, Silanikove N and Maltz E. 2002. Heat stress in lactating dairy cows: a review. *Livestock Production Science* 77, 59-91.
- Kendall PE, Verkerk GA, Webster JR and Tucker CB. 2006. Sprinklers and shade cool cows and reduce insectavoidance behavior in pasture-based dairy systems. *J. Dairy Sci* 90, 3671–3680.
- Köppen W. 1931. *Climatologia*. Fundo de Cultura Econômica, Buenos Aires, Argentina.
- Johnson HD, Ragsdale AC, Berry IL. and Shanklin MD. 1962. Effect of various temperature-humidity combinations on milk productions of holstein cattle. 62th. University of Missouri, Columbia
- Lambertz C, Sanker C and Gauly M. 2014. Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. *Journal of Dairy Science* 97, 319-329.
- Mader TL, Davis MS and Brown-Brandl T. 2006. Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science* 84, 712–719.
- Mitlohner FM, Morrow JL, Dailey JW, Wilson SC, Galyean ML, Miller MF and Mc Glone JJ. 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. *J. Anim. Sci.* 79, 2327–2335
- Morais DA, Maia ASC, Silva RG, Vasconcelos AM and Lima PO. 2008. Variação anual de hormônios tireoideanos e características termorreguladoras de vacas leiteiras em ambiente quente. *Revista Brasileira de Zootecnia* 37, 538-545
- Muller CJC, Botha JA and Smith WW. 1994. Effect of shade on various parameters of Friesian cows in Mediterraneo climate in South Africa. Fed an water intake, milk production an milk composition. *J. Anim. Sci.* 24, 49 – 55.
- Murphy MR, Davis CL and McCoy GC. 1983. Factors affecting water consumption by Holstein cows in early lactation. *J. Dairy Sci.* 66, 35–38.
- Robinson NE. 2004 Homeostase, Termorregulação. In: Cunningham, J. G.; *Tratado de Fisiologia Veterinária*. 3 th. Rio de Janeiro, BR.
- Schütz KE, Cox NR and Tucker CB. 2014. A field study of the behavioral and physiological effects of varying amounts of shade for lactating cows at pasture. *J. Dairy Sci.* 97, 1–7
- Silanikove N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science* 67,1–18.
- Silanikove N, Shapiro F and Shinder D. 2009. Acute heat stress brings down milk secretion in dairy cows by up-regulating the activity of the milk-borne negative feedback regulatory system. *BMC Physiol* 9, 13.

Smith RR, Moreira LVH and Latrille II. 2002. Characterization of dairy productive systems in the tenth region of Chile using multivariate analysis. *Agricultura técnica* 62, 35-395.

Tresoldi G. 2012. Relações sociais entre vacas leiteiras e possíveis consequências na produtividade e bem-estar animal. Dissertação, Programa de Pós-graduação em Agroecossistemas da Universidade Federal de Santa Catarina, Santa Catarina, Brasil.

Tucker CB, Rogers AR, and Shutz KE. 2008. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. *Appl. Anim. Behav. Sci.* 109,141-154.

Vasconcelos JLM and Demetrio DGB. 2011. Manejo reprodutivo de vacas sob estresse calórico. *Revista Brasileira de Zootecnia* 40, 396-401.

West JW, Mullinix BG and Bernard JK. 2003. Effects of Hot, Humid Weather on Milk Temperature, Dry Matter Intake, and Milk Yield of Lactating Dairy Cows. *J. Dairy Sci.* 86, 232–242.

Table I. Values of air temperature, relative humidity and THI measured in the paddocks with and without shade at 11 (GMT-2: 00) and at 17(GMT -2:00)

Day/month	Group	Time (h)	Dry bulb temperature (°C)	Relative humidity (%)	THI
28/01	with shade	11	16.00	80.5	62.00
		17	25.6	56.4	73.46
29/01	no shade	11	19.0	83.4	65.52
		17	27.6	63.5	77.08
29/01	with shade	11	17.6	84.4	63.26
		17	25.4	53.3	72.72
30/01	no shade	11	18.3	80.5	64.28
		17	28.6	41.3	75.51
30/01	with shade	11	17.6	81.7	63.18
		17	27.8	43.3	74.8
31/01	no shade	11	19.7	79.7	66.49
		17	28.7	49.8	76.79
31/01	with shade	11	18.3	84.2	64.40
		17	27.8	55.6	76.37
1/02	no shade	11	20.6	87.4	68.36
		17	30.8	49.9	79.55
1/02	with shade	11	20.4	87.0	68.01
		17	29.7	53.4	78.64

Table II. Value of variables in discrimination between groups of animals from discriminant analysis in behavioral traits of dairy cows with or without access to shade.

Step	Entered	Partial R-Square	Pr > F	Pr > ASCC*
1	Panting Score at 19h	0.6592	<,00001	<.00001
2	Grazing time	0.434	<,00001	<.00001
3	Water ingestion bouts	0.2014	0.0007	<.00001
4	Aggressive interactions	0.174	0.0022	<.00001
5	Respiratory rate	0.1509	0.0058	<.00001
6	Time spent lying	0.1378	0.0101	<.00001
7	Panting score at 15h	0.0982	0.0428	<.00001
8	Events of competition for shade	0.1213	0.0207	<.00001
9	Resting while standing	0.098	0.0453	<.00001
10	Permanence near the water cooler	0.0887	0.0646	<.00001
11	Ruminating while lying	0.0855	0.0749	<.00001

*ASCC – Average Squared Canonical Correlation

Table III. Mean values of behavioral attributes of animals from groups 1, 2 and 3 and *P-values*

Attributes	Group 1 LE* n= 27	Group 2 ME* n=34	Group 3 SE* n=9	P>F
Walking head down (min)	20.37	18.82	8.89	Ns
Walking tall head (min)	7.04	8.53	13.33	Ns
Water ingestion bouts	1.56c	3.85b	6.33a	<0.0001
Total rumination (min)	203.70a	180.59a	90.00b	<0.0001
Ruminating while lying (min)	85.81a	79.41a	38.89b	0.0266
Ruminating while standing (min)	127.44a	104.41ab	63.33b	0.0052
Grazing (min)	177.04b	242.94a	175.56b	<0.0001
Total time resting (min)	195.93b	168.53b	277.78a	<0.0001
Resting while lying (min)	97.04a	59.41b	75.56ab	0.0012
Resting while standing (min)	98.07b	100.59b	202.22a	<0.0001
Total time lying (min)	202.22a	144.12b	120.00b	<0.0001
Total time standing (min)	222.21	215.78	234.76	ns
Seeking for shade (min)	285.19a	148.53b	62.22b	0.0001
Staying near the water trough (min)	47.04b	50.59b	153.33a	<0.0001
Competition near water trough (n°)	0.26	0.18	0.44	ns
Competition for shade (n°)	0.11b	0.15b	1.22a	0.0003
Aggressive interaction (n°)	0.30b	0.65b	2.67a	<0.0001
Milk production (kg/d)	22.81	21.8	19.31	ns
DIM	131.67	127.15	138.44	ns
Parity (n°)	1.93	1.82	2.89	ns
Heart rate (n°)	83.93b	89.71ab	98.33a	0.0106
Respiratory rate (n°)	57.33b	76.12a	84.67a	<0.0001
Rectal temperature (°C)	39.61b	39.66b	40.30a	0.0267

Ruminal movements (n ^o)	2.93a	2.09bc	1.44c	0.0003
Panting score at 15 hours	0.00c	0.59b	2.78a	<0.0001
Panting score at 19 hours	0.19c	1.18b	3.89a	<0.0001

*LE = mild stress * ME = moderate stress * SE = severe stress

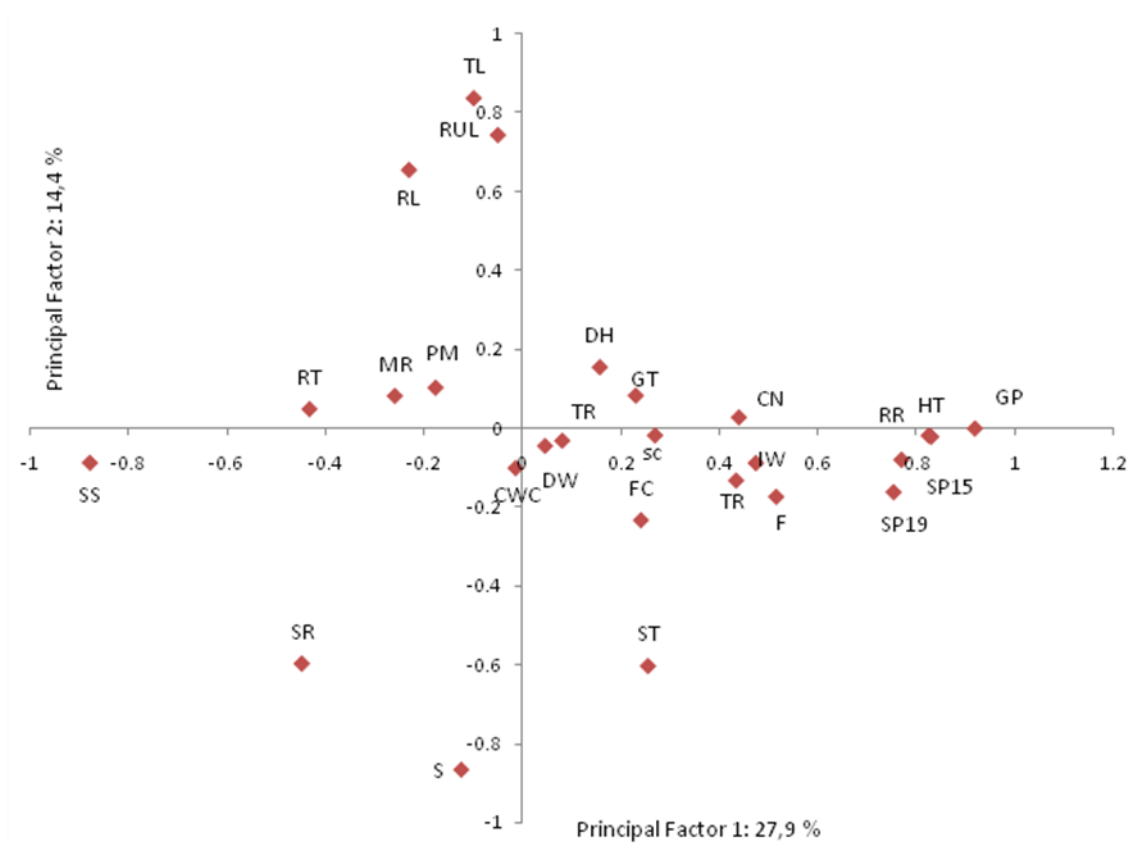


Figure 1. Orthogonal plane for the behavioral attributes of cows under heat stress with and without access to shade. Legend: CN (permanence around the water trough), CWC (competition for water cooler), DW (walking head down), F (aggressive interaction), GP (group), GT (grazing time), HW (walking tall head), IW (water ingestion bouts), MR (ruminal movements), RC (cardiac rate), RL (lying at rest), RR (respiratory rate), RT (ruminating time), RUL (lying while ruminating), S (standing time), SC (competition for shade), SP15 (panting score at 15 hours), SP19 (panting score at 19 hours), SR (ruminating while standing), SS (seeking for shade), ST (resting while standing), TH (heat tolerance), TL (lying total time), TR (rectal temperature), TRE (resting time) and PM (milk production).

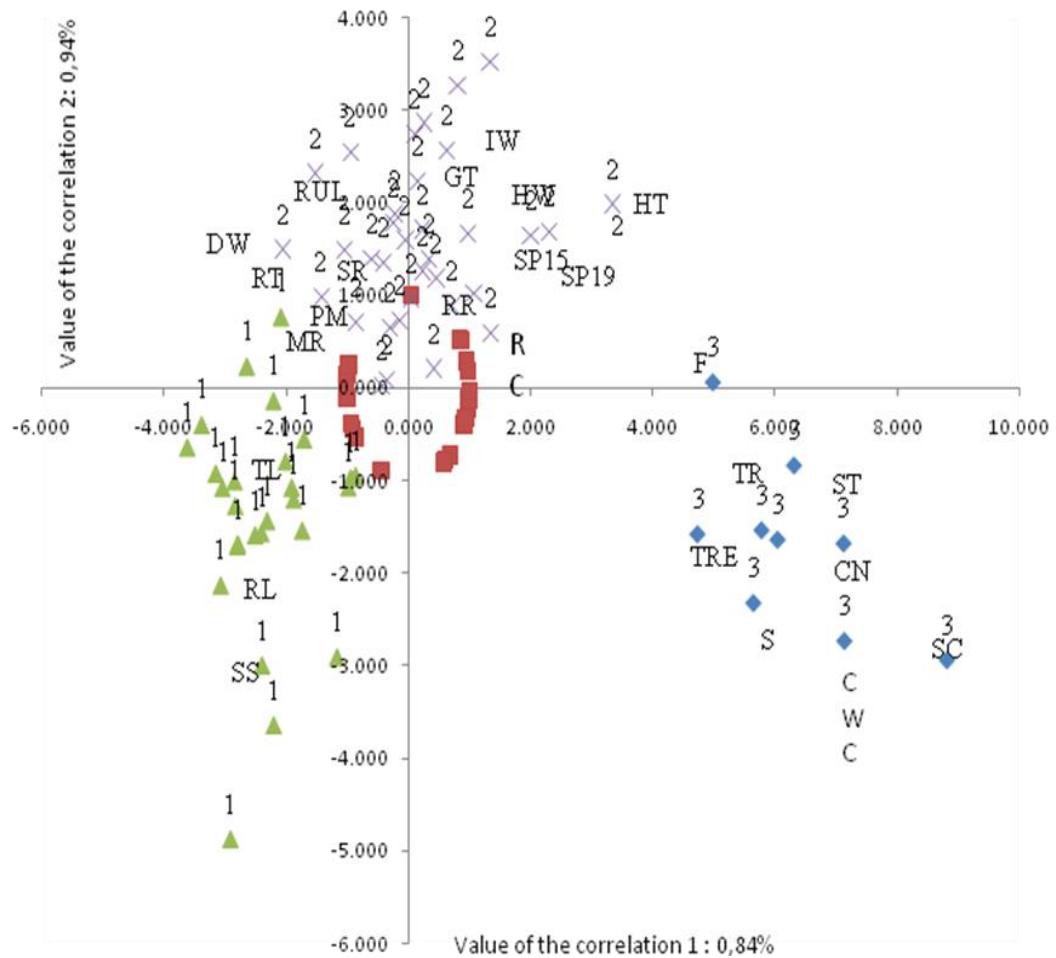


Figura 2 –Canonical analysis of characteristics of behavior and physiological attributes of cows under heat stress with or without access to shade. Legend: CN (permanence around the water trough),CWC (competition for water cooler), DW (walking head down),F (aggressive interaction) ,GP (group), GT (grazing time), HW (walking tall head), IW (water ingestion bouts), MR (ruminal movements), RC (cardiac rate), RL (lying at rest), RR (respiratory rate), RT (ruminating time), RUL (lying while ruminating), S (standing time), SC (competition for shade), SP 15 (panting score at 15 hours), SP 19 (panting score at 19 hours), SR (ruminating while standing), SS (seeking for shade), ST(rest while standing),TH (heat tolerance),TL (lying time),TR (rectal temperature), TRE (resting time) and PM (milk production).

CAPÍTULO III

3.1 CONSIDERAÇÕES FINAIS

Nos últimos anos aceita-se que os animais são seres sencientes, ou seja, indivíduos que tem capacidade de sentir, assim o estudo do comportamento animal se tornou de extrema importância para compreender ações e determinar melhorias nos sistemas criatórios a fim de proporcionar estados positivos de bem-estar animal.

As variáveis comportamentais, sociais e fisiológicas utilizadas neste trabalho caracterizaram os diferentes níveis de estresse térmico enfrentado pelas vacas de leite durante a estação quente em Lages, demonstrando essas variáveis estavam alteradas na maioria dos animais. Apesar de não se ter medido o cortisol plasmático, as observações comportamentais modificadas demonstraram que as vacas se encontravam estressadas, e que a compensação pelo resfriamento noturno observado na produção leiteira não se estendeu a todas as variáveis comportamentais e fisiológicas. Talvez se a duração deste estudo fosse maior, os resultados apresentados no grupo de vacas sem sombreamento seria mais pronunciado em comparação ao grupo com sombreamento, podendo causar alteração na quantidade de leite produzida pelos animais. Como não foi realizado o comportamento noturno, não pode afirmar se as vacas compensaram a ingestão de forragem durante este período.

O clima quente afetou o comportamento animal e os atributos fisiológicos contribuindo para a redução do bem estar animal. A área sombreada apresentou redução de temperatura do bulbo seco de até 3.5°C, assim o uso de sombreamento como o do sistema silvipastoril contribui para minimizar o estresse térmico enfrentado pelas vacas leiteiras, mesmo em clima subtropical de altitude.

3.2 Referências bibliográfica

ABREU, A. S.; FISCHER, V.; KOLLING, G. J. Estresse calórico induzido por privação de acesso à sombra em vacas holandesas reduz a produção leiteira e a estabilidade térmica do leite. In: CONFERÊNCIA INTERNACIONAL DE LECHE INESTABLE, 2., 2011, Colonia del Sacramento. **Anais...** Colonia del Sacramento: [s.n.], 2011. 1 CD-ROM.

ALLEN, J. D. et al. Managing heat stress and its impact on cow behavior. In: WESTERN DAIRY MANAGEMENT CONFERENCE, 2013, Reno, NV. **Proceedings...** Reno, NV: [s.n.], 2013.

ANDERSON, S. D. et al. Effects of adjustable and stationary fans with misters on core body temperature and lying behavior of lactating dairy cows in a semiarid climate. **Journal of Dairy Science**, Champaign, v. 96, n. 7, p. 4738-4750, 2013.

ANTUNES, L. E. C; RASSEIRA, M. C. B. **Aspectos técnicos da cultura da amora preta**. Pelotas: Embrapa Clima Temperado, 2004. 54 p. (Documentos, 122).

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis**. 18th edition. Arlington, VA, USA: AOAC, 2004. 2 vol.

ATRIAN, P.; SHAHRYAR, H.A. Heat stress in dairy cows (a review). **Res. in Zoology**. v 2, p.31-37. 2012.

AZEVÊDO, D. M. R; ALVES A. A. Bioclimatologia aplicada à produção de bovinos leiteiros nos trópicos. Teresina: Embrapa Meio-Norte, 2009. 83 p. (Documentos, 188).

BACCARI, JUNIOR. F. et al. Shade management in tropical environment for milk production in crossbred cows. In: WESTERN SECTION AMERICAN SOCIETY OF AGRICULTURAL ENGINEERING, 1982, [S.I.]. **Proceedings...** [S.I.]: [s.n.], 1982. p. 209-210. v. 33.

BACCARI, JUNIOR. F.; AGUIAR, I. S.; TEODORO, S. M. Comportamento adaptativo termorregulador de vacas holandesas sob radiação solar direta, mediante o aproveitamento de sombra e água. In: CONGRESSO DE ZOOTECNIA, 6., 1997, Lisboa. **Anais...** Lisboa: APEZ, 1997. p. 331-336.

BACCARI, JUNIOR. F. Manejo ambiental para produção de leite em climas quentes. In: CONGRESSO BRASILEIRO DE BIOMETEOROLOGIA, 2., 1998, Goiânia. **Anais...** Goiânia: Universidade Católica de Goiás, 1998. p. 136-161.

BACCARI, JUNIOR. F. **Manejo ambiental da vaca leiteira em clima quente**. Londrina: UEL, 2001, p. 142.

BACH, A. et al. Associations between nondietary factors and dairy herd performance. **J. Dairy Sci.**, Champaign, v. 91, n. 8, p. 3259-3267, 2008.

BAÊTA, F. C.; SOUZA, C. F. **Ambiência em edificações rurais: conforto animal**. Viçosa: UFV, 1997. 246 p.

BAUMGARD, L. H.; RHOADS, R. P. The effects of hyperthermia on nutrient partitioning. In: CORNELL NUTR. CONF., 2007, Ithaca, NY. **Proceedings...** Ithaca, NY: [s.n.], 2007. p. 93-104.

BROWN-BRANDL, T. M. et al. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle. Part 1: analyses of indicators. **Biosyst. Eng.**, v. 90, n. 4, p. 451–462, 2005.

CAMPOS, A. T. et al. Estudo do potencial de redução da temperatura do ar por meio do sistema de resfriamento adiabático evaporativo na região de Maringá-PR. **Acta Scientiarum**, Maringá, v. 24, n. 5, p. 1575-1581, 2002.

COLLIER, R. J.; COLLIER, J. L. **Environmental Physiology of Livestock**. Garsington Road: Published by John Wiley & Sons, Inc, Oxford. 2012.

COLUMBIANO, V. S. **Identificação de QLT nos cromossomos 10, 11 e 12 associados ao estresse calórico em bovinos**. 2007. 60 f. Dissertação (Mestrado em Genética e Melhoramento Animal) - Universidade Federal de Viçosa, Minas Gerais, 2007.

CONCEIÇÃO, M. N. **Avaliação da influência do sombreamento artificial no desenvolvimento de novilhas em pastagens**. 2008. Tese (Doutorado em Agronomia) - Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2008.

CURTIS, S. E. **Environmental management in animal agriculture**. Iowa: Ames the Iowa State University, 1983. 402 p.

DEAG, J. M. **O comportamento social dos animais**. São Paulo: Ed. da Universidade de São Paulo, 1981. (Temas de Biologia, v. 26).

DEITENBACH, A. et al. **Manual agroflorestal para a Mata Atlântica**. Brasília: Ministério do Desenvolvimento Agrário, 2008.

DIKMEN, S.; HENSEN, P. J. Is the temperature-humidity index the best indicator heat stress in lactating dairy cows in a subtropical environment? **Journal of Dairy Science**, Champaign, v. 92, n. 1, p. 109-116, 2009.

DUKES, H. H. **Fisiologia dos animais domésticos**. 11. ed. Rio de Janeiro: Guanabara Koogan, 1996. 856 p.

EDMONSON, A. J et al. A body condition scoring chart for Holstein dairy cows

Journal of Dairy Science, 1989. V.72, p. 68--78.

FUQUAY, J. W.; FOX, P. F.; McSWEENEY, P. L. H. **Encyclopedia of dairy science**. 2. Ed. [S.l.]: Elsevier Ltd, 2011. v. 4, p. 567-574.

GALINDO, F.; BROOM, D. M. The relationships between social behavior of dairy cows and the occurrence of lameness in three herds. **Res. Vet. Sci.**, London. v. 69, n. 1, p. 75-79, 2000.

GAUGHAN, J. B.; DAVIS, M. S; MADER, T. L. Wetting and the physiological responses of grain-fed cattle in a heated environment. **Aust. J. Agric. Res.**, Melbourne, v. 55, n.3, p. 253–260, 2004.

GONÇALVES, L. C.; BORGES, I.; FERREIRA, P. D. S. **Alimentação de gado de leite**. Belo Horizonte: FEPMVZ -Editora, 2009, 412 p.

GRANT, R. Taking advantage of natural behavior improves dairy cow performance. In: WESTERN DAIRY MANAGEMENT CONF., 2007, Reno, NV. **Proceedings...** Reno, NV: [s.n.], 2007. p. 225-236.

HAHN, G. L. Dynamic responses of cattle to thermal heat loads. **Journal of Animal Science**, Albany, v. 77, p. 10-20, 1997.

HAMMAMI, H. et al. Evaluation of heat stress effects on production traits and somatic cell score of Holsteins in a temperate environment. **J. Dairy Science**, Champaign, v. 96, n. 3, p. 1844-1855, 2013.

HÖTZEL, M. J. et al. Effect of water availability on the drinking behaviour and milk production of Holstein cows. In: CONGRESS OF THE INTERNATIONAL SOCIETY FOR APPLIED ETHOLOGY, 2000, Florianópolis. **Proceedings...** Florianópolis: [s.n.], 2000. 145 p.

IGONO, M. O.; BJOTVET, G.; SANFORD-CRANE, H. T. Environmental profile and critical temperature effects on milk production of Holstein cows in desert climate. **Int. J. Biometeorol.** Berlin, v. 36, n. 2, p. 77-87, 1992.

JANUSCKIEWICZ, E. R.; RUGGIERI, A. C.; CAPUTTI, G. P. **Comportamento ingestivo de bovinos em pastejo**. Enciclopédia Biosfera. Goiânia: Centro Científico Conhecer, 2011. v. 7. n. 12.

JOHNSON, H. D. et al. **Effect of various temperature-humidity combinations on milk productions of holstein cattle**. Columbia: University of Missouri, 1962. 39 p.

KADZERE, C. T. et al. Heat stress in lactating dairy cows: a review. **Livestock Production Science**, Amsterdam, v. 77, n. 1, p. 59-91. 2002.

KENDALL, P. E et al. Sprinklers and shade cool cows and reduce insectavoidance behavior in pasture-based dairy systems. **J. Dairy Sci.**, Champaign, v. 90, n. 8, p. 3671–3680, 2006.

KÖPPEN, W. **Climatologia**. México: Fundo de Cultura Econômica, 1931.

LAMBERTZ, C.; SANKER, C.; GAULY M. Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems, **Journal of Dairy Science** 97: 319-329. 2014.

MADER, T. L; DAVIS, M. S; BROWN-BRANDL, T. Environmental factors influencing heat stress in feedlot cattle. **Journal of Animal Science**, Champaign, v. 84, n. 3,p. 712–719, 2006.

MARTELLO, L. S. **Interação animal-ambiente**: efeito do ambiente climático sobre as respostas fisiológicas e produtivas de vacas Holandesas em *free-stall*. 2006. Tese (Doutorado em Qualidade e Produtividade Animal) - Universidade de São Paulo, Pirassununga – SP, 2006.

MATARAZZO, S. V. et al. Intermittência de acionamento do sistema de resfriamento evaporativo em freestall e sua influência no conforto térmico de vacas em lactação. In: REUNIÃO DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 40., 2003, Santa Maria. **Anais...** Santa Maria: UFSM, 2003.

MITLOHNER, F. M, et al. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. **J. Anim. Sci.**, Champaign, v. 79, n. 9, p. 2327–2335, 2001.

MORAIS, D. A. et al. Variação anual de hormônios tireoideanos e características termorreguladoras de vacas leiteiras em ambiente quente. **Revista Brasileira de Zootecnia**, Viçosa, MG., v. 37, n. 3, 2008.

MULLER, C. J. C.; BOTHA, J. A; SMITH, W. W. Effect of shade on various parameters of Friesian cows in Mediterraneo climate in South Africa. Fed an water intake, milk production an milk composition. **J. Anim. Sci.**, South Africa, v. 24, n. 2, p. 49–55, 1994.

MURPHY, M. R.; DAVIS, C. L.; MCCOY, G. C. Factors affecting water consumption by Holstein cows in early lactation. **J. Dairy Sci.**, Champaign, v. 66, n. 1, p. 35–38, 1983.

PAES LEME, T. M. S. et al. Comportamento de Vacas Mestiças Holandesas X Zebu, em Pastagem de *Brachiaria Decumbens* em Sistema Silvipastoril. **Ciência agrotécnica**, Lavras, v. 29, n. 3 p. 668-675, maio/junho, 2005.

PEENINGTON, J. A.; VAN DEVENDER, K. **Heat stress dairy cattle**. UACES publications, 2004.

PEREIRA, C. C. J. **Fundamentos de Bioclimatologia Aplicados à Produção Animal**. Belo Horizonte: FEPMVZ, 2005.

PHILLIPS, C. J.; RIND, M. I. The effects of social dominance on the production and behavior of grazing dairy cows offered forage supplements. **Journal of Dairy Science**, Champaign, v. 85, n. 1, p. 51-59, 2002.

PIRES, M. F. A.; CAMPOS, A. T. **Modificações ambientais para reduzir o estresse calórico em gado de leite**. Juiz de Fora: Empresa brasileira de pesquisa agropecuária, 2004. p. 1-6. (Comunicado técnico, 42).

PORCIONATTO, M. A. F.; et al. Influência do estresse calórico na qualidade e na produção de leite. **Rev. Acad., Ciênc. Agrár. Ambient.**, Curitiba, v. 7, n. 4, p. 483-490, 2009.

PRIVOLO, G.; RIVA E. One year study of lying and standing behaviour of dairy cows in a freestall barn in Italy. **J. Ag. Eng.**, Cidade, v. 2,p. 27-33, 2009.

RHOADS, M. L. et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. **J. Dairy Sci.**, Champaign, v. 92, n. 5, p. 1986-1997, 2009.

ROBINSON, N. E. et al. Homeostase, Termorregulação. In: CUNNINGHAM, J. G. **Tratado de Fisiologia Veterinária**. 3. ed. Rio de Janeiro, RJ: Guanabara Koogan, 2004. p. 550-561.

SCHÜTZ, K.E.; COX, N.R.; TUCKER, C.B. A field study of the behavioral and physiological effects of varying amounts of shade for lactating cows at pasture, **J. Dairy Sci.**, Champaign, v. 97, p.1–7, 2014.

SCHUTZ, K. E. et al. The amount of shade influences the behavior and physiology of dairy cattle, **J. Dairy Sci.**, Champaign, v. 93, n. 1,p. 125–133, 2010.

SCHÜTZ, K. E.; TUCKER, C. B.; ROGERS, A. R. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. **J. Dairy Sci**, Champaign, v. 109, n. 2-4,p. 141-154, 2008.

SELYE, H. A. A Syndrome produced by diverse nocuous agents. **Nature**, v. 138, p. 32, 1936.

SILANIKOVE, N. Impact of shade in hot Mediterranean on feed intake, feed utilization and body fluid tribution in sheep. **Appetite**, v. 9, n. 3, p. 207–215, 1987.

SILANIKOVE, N. Effects of heat stress on the welfare of extensively managed domestic ruminants. **Livestock Production Science**, Amsterdam, v. 67, n. 1-2, p. 1–18, 2000.

SILANIKOVE, N.; SHAPIRO, F.; SHINDER, D. Acute heat stress brings down milk secretion in dairy cows by up-regulating the activity of the milk-borne negative feedback regulatory system. **BMC Physio**, v. 9, n. 13, 2009.

SILVA, R. G. **Introdução à bioclimatologia animal**. São Paulo: Nobel, 2000, 286 p.

SMITH, D. L. et al. Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. *Short communication*, **Journal of Dairy Science**, Champaign, v. 96, n. 5, p. 3028-3033, 2013.

SMITH, J. F. et al. Effect of cross ventilation with or without evaporative pads on core body temperature and resting time of lactating cows. **J. Dairy Sci.**, Champaign, 2012.

SMITH, R. R.; MOREIRA, L. V. H.; LATRILLE L. L. Characterization of dairy productive systems in the tenth region of Chile using multivariate analysis. **Agricultura Técnica**, Santiago, v. 62, n. 3, p. 35-395, 2002.

STARLING, J. M. C. et al. Análise de Algumas Variáveis Fisiológicas para Avaliação do Grau de Adaptação de Ovinos Submetidos ao Estresse por Calor. **Revista Brasileira de Zootecnia**, Viçosa, v. 31, n. 5, p. 2070-2077, 2002.

TAO, S.; DAHL, G. E. Heat stress effects during late gestation on dry cows and their calves. **Journal of Dairy Science**, Champaign, v. 96, n. 7, p. 4079-4093, 2013.

TRESOLDI, G. **Relações sociais entre vacas leiteiras e possíveis consequências na produtividade e bem-estar animal**. 2012. Dissertação (Programa de Pós-graduação em Agroecossistemas) - Universidade Federal de Santa Catarina, Santa Catarina, 2012.

TUCKER, C. B.; ROGERS, A. R.; SCHÜTZ, K. E. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. **Appl. Anim. Behav. Sci.**, Amsterdam, v. 109, n. 2-4, p. 141-154, 2008.

TUCKER C. B.; WEARY, D. M. Bedding on Geotextile Mattresses: How Much is needed to Improve Cow Comfort? **Journal of Dairy Science**, Champaign, v. 87, n. 9, p. 2889-2895, 2004.

VASCONCELOS, J. L. M; DEMETRIO, D. G. B. Manejo reprodutivo de v: sob estresse calórico. **Revista Brasileira de Zootecnia**, Viçosa, v. 40, p. 396-401, 2011.

WEST, J. W.; MULLINIX, B. G ; BERNARD, J. K. Effects of Hot, Humid weather on Milk Temperature, Dry Matter Intake, and Milk Yield of Lactating Dairy Cows.

J. Dairy Sci., Champaign, v. 86, n. 1, p. 232–242, 2003.

WEST, J. W. et al. Effects of dietary fiber on intake, milk yield, and digestion by lactating dairy cows during cool or hot, humid weather. **J. Dairy Sci.**, Champaign, v. 82, n. 1, p. 2455–2465, 1999.

WHEELLOCK, J. B. et al. Effects of heat stress on energetic metabolism in lactating Holstein cows. **J. Dairy Sci.**, Champaign, v. 93, n. 2, p. 644–655, 2010.

ZIMBELMAN, R. B. et al. A re-evaluation of the impact of temperature humidity index (THI) and black globe temperature humidity index (BGHI) on milk production in high producing dairy cows. In: SOUTHWEST NUTRITION AND MANAGEMENT CONFERENCE, 24., 2009, Tempe, AZ. **Proceedings...** Tempe, AZ: [s.n.], 2009. p. 158-168.

4.3 Apêndice

Animal
An International Journal of Animal Bioscience
Instructions for authors
Last updated November 2013

Recommendations for preparation of papers

The responsibility for the preparation of a paper in a form suitable for publication lies in the first place with the author. Authors should consult a free issue or a free article of *animal*, available at <http://journals.cambridge.org/anmsample>, in order to make themselves broadly familiar with the layout and style of *animal*. The English must be acceptable for publication. If the English is not good enough, editors may ask for a linguistic revision by a third-party service at any stage of the review process and at the author's cost. The copyeditor will check and correct minor grammatical errors and journal styles in the accepted manuscripts, but he will not perform language editing. A variety of third-party services specialising in language editing and/or translation can be found here: <http://journals.cambridge.org/action/stream?pageld=8728&level=2&menu=Authors&pageld=3608>. Manuscripts should be prepared using a standard word processing program, presented in a clear, readable format with easily identified sections and headings and typed with double-line spacing with wide margins (2.5 cm). The use of small paragraphs with less than 8 lines must be minimised. The lines must be continuously numbered (on left side); the pages must also be numbered. Font Arial 12 should be used for the text, and Arial 11 for tables and references, in order to easily evaluate manuscript length. The typographical and other conventions to be adopted are set out below. A style sheet is available on our website in order to help the authors to organise their manuscript and to comply with *animal* style format. Manuscripts which do not follow the below mentioned conventions will be sent back to the author.

Title

A title needs to be concise and informative. It should:

- (a) arrest the attention of a potential reader scanning a journal or a list of titles;
- (b) provide sufficient information to allow the reader to judge the relevance of a paper to his/her interests and whether it will repay the effort of obtaining a copy;
- (c) incorporate keywords or phrases that can be used in indexing and information retrieval, especially the animal species on which the experiment has been carried out;
- (d) avoid inessentials such as 'A detailed study of ...', or 'Contribution to ...';
- (e) not include the name of the country or of the region where the experiment took place;
- (f) be shorter than 170 characters including spaces.

Authors and affiliation

The names and affiliations of the authors should be presented as follows:

J. Smith^{1,a}, P.E. Jones², J.M. Garcia^{1,3} and P.K. Martin Jr²

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Animal, Facultad de Veterinaria, Universidad de Zaragoza, C. Miguel Servet, 177, 50013, Zaragoza, Spain

a Present address: Dairy Science Laboratory, AgResearch, Private Bag 11008, Palmerston North, New Zealand (for any author of the list whose present address differs from that at which the work was done)

Corresponding author: John Smith. E-mail: John.Smith@univ.co.uk

The corresponding author indicated in the manuscript who will be the correspondent for a published paper can be different from the corresponding author who submits and manages the manuscript during the review process; the latter corresponding author will need to be registered on Editorial Manager.

Running head

Authors should propose a running head of no more than 50 characters. If the proposed running head is not appropriate, it could be modified by the Editorial Office, with the author's agreement.

Abstract

Every paper should have a one-paragraph abstract of not more than 400 words which is complete and understandable without reference to the paper. It should state succinctly the problem, the experimental methods, results and conclusions but should not be overburdened by numerical values or probability values. References to tables and figures, and undefined abbreviations are not acceptable.

Keywords

Up to a maximum of five keywords selected from CAB Thesaurus (1995) or from an equivalent volume should be selected. Keywords are essential in information retrieval and should be indicative of the content of the paper (animal species, etc.). If the proposed keywords are not appropriate, the manuscript will be returned to the authors. The use of non-standard abbreviations in the list of keywords is discouraged. Implications

Authors must write maximum 100 words explaining the implications of their work. Implications explain the expected importance or economic, environmental and/or social impact. This must be in simple English suitable for non science readers. This section is mandatory and will be peer-reviewed.

Introduction

The Introduction should briefly present the current issues that the authors are addressing while outlining the context of the work, ensuring that the objectives are clearly defined, and that the main features of the experiment or of the work are clear to the reader. Increasing the knowledge on a subject is not an objective per se. References in the Introduction should be limited as it should not be a preliminary discussion or a literature review.

Material and methods

Material and Methods should be described in sufficient detail within this section, so that it is possible for others to repeat the experiment. If the methods are numerous, authors should refer to one of their previous publications in which they are described in detail.

If a proprietary product is used as a source of material in experimental comparisons, this should be described using the appropriate chemical name. If the trade name is helpful to the readers, provide it in parentheses after the first mention. Authors who have worked with proprietary products, including

equipment, should ensure that the manufacturers or suppliers of these products have no objections to publication if the products, for the purpose of experimentation, were not used according to the manufacturer's instructions.

Results - Discussion

They can be presented together (Results and Discussion) or in 2 different sections (Results followed by Discussion). Conventions for presenting these sections or the Materials and Methods section (sub-headings, etc.) are presented later in this document.

Statistical treatment of results

A statistical guide for authors is available on the website at http://www.animal-journal.eu/statistical_instructions.htm. The methods and models of statistical analysis must be indicated and sufficient statistical details given to allow replication of the experiment. Where reference is made to statistical significance, the level of significance attained should normally be indicated by using the following conventional standard abbreviations (which need not be defined): $P > 0.05$ for non-significance and $P < 0.05$, $P < 0.01$ and $P < 0.001$ for significance at these levels. In tables, levels of significance should be indicated by *, ** and *** respectively. Statistical significance (e.g. $P = 0.07$) can also be used in the text or tables. Treatment means should normally be given without their standard deviation (i.e. variability in a sample or a population). An indicator of the precision of the measure such as the pooled standard error, the residual standard deviation (RSD) or the root mean square error (RMSE) should be given for each criteria/item/variable/trait in an additional column (or line). Differences between treatments (or comparison of mean values) will be indicated using the following conventional standard: a, b for $P < 0.05$; A, B for $P < 0.01$; in most cases, the 0.05 level is sufficient.

Tables

These should be as simple and as few as possible. The same material should not normally be presented in tabular and graphical form. When both forms are possible, authors should present tables. Generally, variables are in rows and treatments in columns. In designing tables, authors should refer to the page size and column widths of animal as guidance. Each table should be typed, preferably in double spacing, on a page separate from the main body of the text (one table per page) and an indication given in the text where it should be inserted. Tables which are created in Word should not use tabs but should use the table function within the programme. Tables should not be prepared with vertical lines between columns and horizontal lines between rows of data. Tables should be given Arabic numbering and each should have its own explanatory title, footnotes and definitions of abbreviations which are sufficient to permit the table to be understood without reference to the text ("Effect of fat source and animal breed on carcass composition in pigs" is preferred to "Carcass composition"). The title should not contain too many details about the protocol, the definition of abbreviations, etc. Such details are preferred as footnotes. All tables must be cited consecutively in the text. Column headings should be concise and units should be clearly stated using standard abbreviations; any non-standard abbreviation should be defined. Only the first letter of the first word is in capitals. Sub-items describing the data in the rows

should be indented relative to crossheadings; where they involve printing on more than one line, they should be indented in the second and subsequent lines. Sub-sub-items should also be indented. Footnotes should be used sparingly and kept brief. They must provide the bases for statistics (levels of significance, statistical model, etc.). The reference symbols used in footnotes are numbers in low cases. The values in the tables should be given with meaningful decimals; practically, the last digit should correspond to about one tenth of the standard error. The number of decimals for mean treatment values and the corresponding indicator of residual variability (RSD, SEM, RMSE, etc.) should be consistent in all the tables, either identical or one more for the variability indicator, but not both possibilities. Values such as efficiencies or digestibilities are preferred as percentages.

Figures

It is recommended that the width of any diagram submitted should be either 175 mm (2 columns) or 83 mm (1 column) including the legend at the side(s). In choosing ornaments, solid symbols should be used before open symbols, and continuous lines before dotted or dashed lines. The size of symbols should be appropriate (neither too small nor too big and clumsy). The use of colour in figures should be avoided, unless it is essential to understanding the figure. Figures are then usually supplied as black and white and as one file per figure. Colour figures are reproduced at no cost to the author for the on-line version. But the authors are liable to cover the additional costs for printing figures in colour. Publication charges can be found at http://www.animal-journal.eu/documents/Reprints_cost.pdf. All figures must be numbered consecutively in the text. Captions for all figures should be typed on a separate page at the end of the manuscript and should be sufficiently detailed to allow the figure to be understood without reference to the text; figures are submitted without their captions. An indication of where a figure should appear should be given within the text. Diagrams and plates are referred to within the text as Figure 1, etc., and the captions begin with Figure 1, etc. For details of submission requirements, refer to section on 'Submission and evaluation of the manuscript'.

Acknowledgements

In this section, the authors may acknowledge (briefly) their support staff, their funding sources, their credits to companies or copyrighted material, etc. All papers with a potential conflict of interest must include a description/explanation under the Acknowledgements heading

References

It is the author's responsibility to ensure that all references are correct. The references should adhere to the guidelines, be relevant to the text content and they should all be cited in the text. The maximum number of references is 10 for short communications, 35 for original articles and 50 for review papers, except when the editor agrees a higher number. No more than 3 references can be given for the same statement (except for reviews and meta-analyses). Authors should minimise the number of references to conference proceedings, reports, PhD theses, and other references which cannot easily be obtained by the reader. The accuracy of the references is the responsibility of the authors. Authors should carefully check authors' surnames and first names, article title,

journal title, volume and page numbers, book publisher's information, proceedings exact description. Literature cited should be listed in alphabetical order of authors and references should not be numbered. For a same first author, the rank of references will be i) publications with one author ranked by year; ii) publications with two authors ranked by year; iii) publications with more than two authors ranked by year then, if necessary, by alphabetical order of the second author.

Typical references are:

Journal article or abstract:

Format: Author(s) surname and Initials Year. Title. Full title of the journal volume, pages. The issue within the volume is not mentioned, except if the numbering is per issue and not per volume (ex: newspapers). The word 'abstract' if applicable is not mentioned. Titles which cannot be written in Latin characters will be translated in English, followed by (in xxx) where xxx is the original language.

Examples:

Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to the farm scale. *Animal* 4, 351-365.

Morgavi DP, Martin C, Jouany JP and Ranilla MJ 2012. Rumen protozoa and methanogenesis: not a simple cause-effect relationship. *British Journal of Nutrition* 107, 388-397.

When the article is online but not yet printed, the right format is: Zamaratskaia G and Squires EJ. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal*, doi:10.1017/S1751731108003674, Published online by Cambridge University Press 17 December 2008.

Book:

Format: Author(s) or editor(s) surname and Initials, or institution Year. Title, number of volumes if more than 1, edition if applicable. Name of publisher, place of publication (i.e. city, state (if applicable) and country).

Example: Association of Official Analytical Chemists 2004. *Official methods of analysis*, 2 vol., 18th edition. AOAC, Arlington, VA, USA.

Book chapter or edited conference proceedings:

Format: Author(s) surname and Initials Year. Title. In Title of the book or of the proceedings (eds followed by the editor(s)), volume number when applicable, pages. Name of publisher, place of publication (i.e. city, state (if applicable) and country).

For edited proceedings, it is not necessary to mention the date and place of the symposium.

Example: Nozière P and Hoch T 2006. Modelling fluxes of volatile fatty acids from rumen to portal blood. In *Nutrient digestion and utilization in farm animals* (eds E Kebreab, J Dijkstra, A Bannink, WJJ Gerrits and J France), pp. 40- ... CABI Publishing, Wallingford, UK.

Report at an event (conference, meeting, etc) not included in a book or edited proceedings:

Format: Author(s) surname and Initials Year. Title. Nature of the event, date of the event (i.e. day month year), place of the event (i.e. city, state (if applicable) and country), pages or poster/article number (if applicable).

Examples: Martuzzi F, Summer A, Malacarne M and Mariani P 2001. Main protein fractions and fatty acids composition of mare milk: some nutritional remarks with reference to woman and cow milk. Paper presented at the 52nd Annual Meeting of the European Association for Animal Production, Budapest, 26-29 August 2001, Budapest, Hungary

Bispo E, Franco D, Monserrat L, González L, Pérez N and Moreno T 2007. Economic considerations of cull dairy cows fattened for a special market. In Proceedings of 53rd International Congress of Meat Science and Technology, 5-10 August 2007, Beijing, China, pp. 581–582.

Thesis:

Format: Author surname and Initials Year. Title. Type of thesis, University with English name, location of the University (i.e. city, state (if applicable) and country).

Example: Vlaeminck B 2006. Milk odd- and branched-chain fatty acids: indicators of rumen digestion for optimisation of dairy cattle feeding. Thesis PhD, Ghent University, Ghent, Belgium.

Website addresses can be used when no other reference is available. They should be presented as for standard references but, in addition, they should include the date when the document was retrieved:

Bryant P 1999. Biodiversity and Conservation. Retrieved on 4 October 1999, from <http://darwin.bio.uci.edu/~sustain/bio65/Titlepage.htm>

Citation of references

In the text, references with three or more authors should be cited on all occasions with the first author followed by *et al.* (in italics; e.g. Smith *et al.*). References with two authors should be cited in full on all occasions. Names of organizations used as authors (e.g. Agricultural and Food Research Council) should be written out in full in the list of references and on first mention in the text. Subsequent mentions may be abbreviated (e.g. AFRC). Ampersands (&) are not to be used. Multiple references should be as follows: Wright *et al.*, 1993 and 1994; Wright *et al.*, 1993a and 1993b. When several references are cited simultaneously, they should be ranked by chronological order (e.g. Smith *et al.*, 1995; Fabre *et al.*, 1996; Schmidt *et al.*, 1998; Fabre *et al.*, 1999).

'Personal communication' or 'unpublished results' should follow the name of the author in the text where appropriate. The author's initials but not his title should be included, and such citations are not needed in the reference list. Check that all of the references in the text are in the list of references and vice versa.

Bibliographic database softwares can be used. The output styles for Endnote, Procite and Reference Manager may be found on the journal website http://animal-journal.eu/instructions_to_authors.htm. Supplementary material

Authors can include supplementary material in any type of text (full research paper, review paper, short communication, etc.). Supplementary material will appear only in the electronic version, and is not limited in length. It will be peer-reviewed along with the rest of the manuscript, but will not be copy-edited. Authors are entirely responsible for its content and must check carefully the format and styles. This supplementary material could contain original modus operandi, tables or figures which are not necessary for understanding the text within the main body of the paper, mathematical models, references of publications which are used, for example, in a meta-analysis and which do not

appear in the text, or pictures improving the understanding of the text. The manuscript must stand alone without the supplementary material for those readers who will be reading the hard copy only. This should be submitted with the main manuscript in a separate file and identified as "Supplementary File – for Online Publication Only". Each figure should have its own title embedded in the figure (below). Supplementary material should be identified and mentioned in the main text as Supplementary Table S1, Supplementary Table S2, etc. for tables or Supplementary Figure S1, Supplementary Figure S2, etc. for figures or Supplementary Material S1, Supplementary Material S2, etc. for other material). For example: "The list of references used for the meta-analysis is given in Supplementary Material S1". A link to this on-line supplementary material will be included by the Production Editor at the proof preparation stage.

Typographical conventions and consistencies

Headings

As illustrated and detailed above and in the style sheet (see website), the animal convention is as follows.

(a) Title of the paper is in bold with only the first letter in capitals. Authors' names are in lower case with initial capitals and their addresses are in italics.

(b) Main section headings (Abstract, Introduction, Implications, Material and methods, Results, Discussion, Acknowledgement(s), References) are printed in bold throughout and placed by the left margin.

(c) Subheadings are italicized and only the initial letter is in capitals. The two classes are:

(i) side italics unpunctuated (shoulder headings);

(ii) italics, punctuated and text run-on (side headings).

The sequence is always (i) to (ii).

Abbreviations

When abbreviations are defined in the text, they should be written in bold capitals at first occurrence.

The abbreviations listed below do not require the full spelling.

ACTH Adrenocorticotropic hormone

ADF Acid detergent fibre

ADL Acid detergent lignin

ADP Adenosine diphosphate

ANOVA Analysis of variance

ATP Adenosine triphosphate

BLUP Best linear unbiased prediction

BW Body weight

CoA Coenzyme A

CP Crude protein

DNA Deoxyribonucleic acid

ELISA Enzyme-linked immunosorbent assay

FSH Follicle-stimulating hormone

GLC Gas-liquid chromatography

GLM General Linear Model

HPLC High performance (pressure) liquid chromatography

IGF Insulin-like growth factor

IR	Infrared
LH	Luteinising hormone
MS	Mass spectrometry
n	Number of samples
NAD	Nicotinamide adenine dinucleotide

The names of the chemicals do not need to be written out in full; chemical symbols are sufficient. Fatty acids are abbreviated using the following rules: cis-18:1 for the sum of cis octadecenoic acids. When isomers are described, the double bond positions are identified by numbering from the carboxylic acid end: c9,t11-18:2; iso-15:0. The terms “omega 3” and “omega 6” are banned and replaced by “n-3” and “n-6”, e.g. 18:3n-3. Trivial names can be used for the most known fatty acids (myristic, palmitic, oleic, linoleic, linolenic) and abbreviations in some cases: CLA for conjugated linoleic acids, EPA for eicosapentaenoic acid, DHA for docosahexaenoic acid. Chemical names and trivial names cannot be mixed in a same table.

Capitals

- (a) Initial capitals are used for proper nouns, for adjectives formed from proper names, for generic names and for names of classes, orders and families.
- (b) Names of diseases are not normally capitalized.

Italics

Use italics for:

- (a) titles of books and names of periodicals in the text;
- (b) authors' addresses;
- (c) subheadings (see above);
- (d) titles for tables (but not captions for figures);
- (e) most foreign words, especially Latin words, e.g. ad hoc, ad libitum, et al., in situ, inter alia, inter se, in vitro, per se, post mortem, post partum but no italics for c.f., corpus luteum, e.g., etc., i.e., N.B., via
- (f) mathematical unknowns and constants;
- (g) generic and specific names;
- (h) letters or numbers in the text which refer to corresponding letters or numbers in an illustration;
- (i) letters used as symbols for genes or alleles e.g. HbA, Tf D (but not chromosomes or phenotypes of blood groups, transferrins or haemoglobins, e.g. HbAA, TfDD);
- (j) first occurrence of a special term;
- (k) repeated emphasis of a special term (use cautiously);
- (l) Latin names of muscles (but not of bones), e.g. m. biceps femoris.

Spelling

All papers must be written in English. Spelling may be in British or American English but must be consistent throughout the paper. Please refer to standard dictionaries e.g. Webster's, Collins, Concise Oxford for the correct spelling of words and to Fowler's Modern English Usage (3rd edition, edited by R.W. Burchfield, Oxford University Press) for usage. Care should be exercised in the use of agricultural terminology that is ill-defined or of local familiarity only.

Numerals (a) In text, use words for numbers zero to nine and figures for higher numbers. In a series of two or more numbers, use figures throughout irrespective of their magnitude.

- (b) Sentences should not, however, begin with figures.
- (c) For values less than unity, 0 should be inserted before the decimal point.
- (d) For large numbers in the text substitute 10ⁿ for part of a number (e.g. 1.6 10⁶ for 1 600 000).
- (e) To facilitate the reading of long numbers in tables, the digits should be grouped in three about the decimal sign but no point or comma should be used.
- (f) The multiplication sign between numbers should be a cross (x).
- (g) Division of one number by another should be indicated as follows: 136/273
- (h) Use figures whenever a number is followed by a standard unit of measurement (e.g. 100 g, 6 days, 4th week).
- (i) Use figures for dates, page numbers, class designations, fractions, expressions of time, e.g. 1 January 2007; type 2.
- (j) Dates should be given with the month written out in full in the text and with the day in figures (i.e. 12 January not 12th January). Single non-calendar years should be written 2006/07; periods of two calendar years as 2006-07.
- (k) For time use 24-h clock, e.g. 0905 h, 1320 h.

Units of measurement

The International System of Units (SI) should be used. A list of units is found for example at <http://physics.nist.gov/cuu/Units/units.html>. Recommendations for conversions and nomenclature appeared in *Proceedings of the Nutrition Society*, 31: 239-247, 1972. Some frequently used units which are not in the SI system are accepted: l for litre, ha for hectare, eV for electron-volt, Ci for curie. Day, week, month and year are not abbreviated.

A product of two units should be represented as N•m and a quotient as N/m (e.g. g/kg and not g.kg⁻¹), except in case of two quotients (e.g. g/kg per day and not g/kg/day).

Concentration or composition

Composition expressed as mass per unit mass or mass per unit volume should have as denominator the unit of mass, the kilogram, or the unit of volume, the litre. Values should thus be expressed as nanograms, micrograms, milligrams or grams per kilogram or per litre. The term content should not be used for concentration or proportion.

Statistical terms

chi square χ^2

coefficient of determination R^2

coefficient of variation CV

correlation

multiple R

sample coefficient r

degrees of freedom d.f.

expectation of mean square e.m.s.

least significant difference LSD

mean square m.s.

non-significant $P > 0.05$

probability P

$P < 0.05$, in tables use *

$P < 0.01$, in tables use **

$P < 0.001$, in tables use ***

regression coefficient b
root mean square error r.m.s.e.
standard deviation s.d.
standard error of difference s.e.d.
standard error of mean s.e.m. j
standard error of estimate $S_{y.x}$
residual standard deviation r.s.d.

4.4 Vita

Elissa Forgiarini Vizzotto, filha de Gilberto Vizzotto e Carmem Forgiarini Vizzotto, brasileira nascida em Faxinal do Soturno, Rio Grande do Sul, no dia 12 de janeiro de 1987.

Realizou o ensino fundamental na Escola Estadual Dom Antonio Reis – Faxinal do Soturno e o ensino médio no Colégio Metodista Centenário – Santa Maria. Em 2001 entrou para o curso Técnico Agrícola habilitação em agropecuária concomitante, no Colégio Agrícola de Santa Maria concluindo em 2005. No ano 2007 iniciou o curso de Zootecnia na Universidade Federal de Santa Maria, sendo concluído em julho de 2011.

Durante os anos de faculdade realizou estágio no laboratório de bovino de leite. O estágio final de curso foi realizado na Central da Cooperativas Gaúchas LTD. (CCGL), no tambo experimental realizando pesquisas a serviço da demanda dos produtores e na assistência técnica a produtores de leite.

Em abril de 2012 iniciou o curso de Mestrado no Programa de Pós-Graduação em Zootecnia UFRGS.