UNIVERSIDADE FEDERAL DO PARANÁ

JÉSSICA CARVALHO SEABRA

DIFERENTES INTENSIDADES DE EXERCÍCIO PARA O BEM-ESTAR DE CAVALOS DE CORRIDA EM TREINAMENTO

CURITIBA

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Curitiba, 14 de Dezembro de 2017.

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"Dificuldades preparam pessoas comuns para destinos extraordinários."

Clive Staples Lewis

RESUMO

O confinamento é um regime incompatível com o comportamento natural dos cavalos e a imposição desse tipo de manejo pode trazer restrição severa de seu bem-estar, podendo causar aparecimento de distúrbios comportamentais, alterações hormonais e diminuição da imunidade. Várias pesquisas relacionadas à medicina humana revelam que a prática de exercício físico pode ser capaz de reduzir significativamente os níveis de ansiedade. Sendo assim, o objetivo desse estudo foi avaliar o bem-estar de cavalos de corrida mantidos em regime de confinamento em baias, quando submetidos à diferentes intensidades de exercício. Este experimento utilizou 10 éguas e 6 garanhões da raça puro-sangue inglês alojados em baias individuais 24 horas por dia no Jockey Club do Paraná, em Curitiba, Brasil. Os cavalos tinham cerca de 2 anos de idade e compartilhavam a mesma rotina de exercícios que foi dividida em 4 níveis crescentes de intensidade. As análises comportamentais e coletas de sangue foram realizadas em cada nível de exercício para mensurar o índice de prevalência de comportamentos anormais e para medir o nível de imunidade dos animais respectivamente. No último dia de treinamento antes da primeira corrida oficial, a câmera termográfica foi utilizada para coletar imagens do olho esquerdo dos cavalos antes e após o exercício para aferição da temperatura máxima do globo ocular. O experimento foi realizado em delineamento inteiramente casualizado com 16 repetições. A análise dos dados comportamentais foi feita através de estatística descritiva e pela técnica de mineração de dados, enquanto a mudança na relação de neutrófilos/linfócitos, a diferença no número total de leucócitos e o aumento da temperatura ocular foram analisados com a ANOVA e teste de Bartlettt, sendo posteriormente submetidos ao teste de Tukey. Análises de correlação entre a frequência respiratória, Índice de Conforto Térmico e temperatura ocular máxima também foram realizadas. A árvore de decisão gerada pelo modelo após o procedimento de mineração de dados mostrou que alguns comportamentos anormais (ingerir cama, atacar o ar e escoicear a baia) foram mais frequentes na primeira intensidade de exercício. Também foi observado aumento significativo ($P < 0,01$) na relação Neutrófilos/Linfócitos durante o treinamento mais intenso e a primeira corrida oficial, além do aumento significativo ($P < 0,01$) no número total de leucócitos após a primeira corrida oficial. Embora as diferentes intensidades de treinamento tenham influenciado significativamente o leucograma, todos os resultados estavam dentro dos parâmetros fisiológicos normais. Também houve diferença significativa ($P < 0,01$) entre as medidas da temperatura do olho realizadas antes e depois a sessão de treinamento, havendo uma correlação linear positiva entre o aumento da temperatura máxima ocular e o aumento da frequência respiratória. Sendo assim, conclui-se que o protocolo de treinamento com exercícios físicos de alta intensidade não gerou alterações negativas nos leucogramas dos animais e foi capaz de diminuir a prevalência de alguns comportamentos anormais, o que parece ter sido benéfico, dentro dos parâmetros analisados. A câmera termográfica também demonstrou um grande potencial para aferição dos níveis de estresse dos animais durante a prática de exercício físico, sendo necessário apenas alguns ajustes para servir adequadamente a essa função.

Palavras-chave: estresse, comportamento, intensidade de exercício, parâmetros hematológicos, equino.

ABSTRACT

The confinement incompatible with the natural behavior of horses and the imposition of this type of management can severely decrease their welfare level, being able to cause behavioral disorders, hormonal changes and decreased immunity. Several researches in the field of human health reveal that the practice of physical exercise can reduce significantly the levels of anxiety. Therefore, the aim of this trial was to evaluate the welfare of racehorses kept confined in individual stalls when submitted to different exercise intensities. This study used 10 mares and 6 stallions of thoroughbred horses housed in individual stalls 24 hours per day at the Jockey Club of Paraná, in Curitiba, Brazil. The horses were about 2 years old and shared the same exercise routine which has been divided into 4 increasing levels of intensity. Behavioral analyzes and blood samples were made during each exercise level to measure the prevalence rate of abnormal behaviors and to measure the immunity level of the animals, respectively. At the last day of training before the horses' first official race, the thermal camera was used to collect images from their left eye before and after the exercise for the measurement of the eyeball maximum temperature. The experiment was carried out in a completely randomized design with 16 replicates. Behaviors were analyzed using descriptive statistic using the data mining technique, in the meanwhile, the change in the neutrophil/lymphocyte ratio, the difference in the total leukocyte and the increase of the eye temperature were analyzed thru ANOVA procedure and the Bartlett test, with post-hoc Tukey test. Correlation analyzes between respiratory rate, Thermal Comfort Index and maximum ocular temperature were also performed. The decision tree generated by the model after the data mining procedure has shown that some abnormal behaviors (bed-eating, attacking the air and box kicking) were more frequent in the light exercise intensity. There was a significant ($P<0.01$) increase in Neutrophil/Lymphocyte ratio during the intense exercise training and the first official race significant ($P<0.01$) increase in the total leukocytes number could also be observed after the first official race. Although the different training intensities have significantly influenced the leukogram, all the results were enclosed in the normal physiological parameters. There was also a significant difference ($P<0.01$) between the eye temperature measurements taken before and after the training session, in the meanwhile, there was also a positive linear correlation between the increase in the maximum ocular temperature and the increase in respiratory rate. Therefore, can be conclude that the training protocol with high intensity exercises had not negative influences on the leukogram of the animals and it was able to reduce the prevalence of some abnormal behaviors, which seems to have been beneficial within the analyzed parameters. Although the thermal camera still needs some adjustments, it has shown great potential for measuring the stress levels of animals during physical exercise.

Keywords: stress, behavior, exercise intensity, hematological parameters, equine.

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1 INTRODUÇÃO

Os cavalos são animais gregários, sociáveis e que em seu ambiente natural, podem percorrer grandes distâncias à procura de alimento. Ao longo da história, os equinos foram domesticados e suas funções foram alteradas com o tempo, sendo atualmente utilizados principalmente como força de trabalho e como atletas em competições equestres.

Os cuidados com a alimentação, a crescente valorização econômica da espécie, bem como a necessidade de criar esses animais em espaço reduzido e muitas vezes urbanizado, vêm contribuindo para o aumento do número de estabelecimentos que optam por manter os animais estabulados. Entretanto, é preciso ressaltar que o confinamento é um regime incompatível com o comportamento natural da espécie equina e que a imposição desse tipo de manejo pode trazer restrição severa de seu bem-estar, tais como alterações psíquicas, físicas e metabólicas que serão prejudiciais à saúde do animal. O estresse oriundo do confinamento em baias é uma das principais causas do aparecimento de distúrbios comportamentais em equinos (GONTIJO, 2010), como as estereotipias, podendo causar também alterações hormonais e consequente diminuição da imunidade.

Várias pesquisas relacionadas à medicina humana revelam que a prática de exercício físico pode ser excelente ferramenta capaz de reduzir significativamente os níveis de ansiedade (LONG e HANEY, 1988; LONG E VAN-STAVEL, 1995; McAULEY,1996). Entretanto, a atividade física excessiva também é vista como uma fonte de estresse, podendo até mesmo ser capaz de induzir à imunossupressão, contribuindo para o aumento da incidência e gravidade de algumas doenças observadas em cavalos de corrida e de alto rendimento (HAIN et al., 1996).

Sendo assim, se faz necessária a realização de estudos que objetivem avaliar o bem-estar de equinos mantidos em regime de confinamento em baias, quando submetidos a diferentes intensidades de exercício.

2 REVISÃO DE LITERATURA

2.1 HISTÓRIA DO CAVALO

A evolução do cavalo começou há cerca de 65 milhões de anos. Entretanto, a domesticação do cavalo pode ser considerada um evento relativamente recentemente, quando comparada ao tempo evolutivo. Os primeiros indícios da domesticação dos equinos foram encontrados em Dereivka, na Ucrânia, e datam de cerca de 6000 anos atrás (LEVINE, 1999). Por esse motivo, o comportamento do cavalo ainda não demonstra muitos sinais de ter sido alterado pela domesticação, sendo essa afirmação evidente quando observamos a facilidade com que esses animais são capazes de voltar ao seu estado feral quando libertos em ambientes desfavoráveis (GOODWIN, 2002).

Os primeiros equinos domesticados eram inicialmente utilizados como fonte de alimento, sendo mantidos em grupos, dentro de uma área restrita de seu ambiente natural. No entanto, com o passar do tempo, o papel exercido pelo cavalo dentro da cultura humana mudou e foi diversificado, sendo a domesticação responsável por restringir muitos aspectos do comportamento natural do cavalo. No primeiro milênio a.c., a importância do cavalo na cultura humana como montaria já havia sido estabelecida. Desde então, o cavalo teve papel fundamental nas mudanças que ocorreram na sociedade humana, sendo utilizado como força de trabalho, ferramenta de guerra, e hoje em dia, como um animal atleta e de estimação. Essas mudanças da sociedade ocorreram em um período muito curto de tempo evolutivo, tornando difícil a adaptação do cavalo (GOODWIN, 2002).

2.2 BEM-ESTAR DO CAVALO ATLETA

2.2.1 Definição de bem-estar animal

O bem-estar animal é definido como o estado do animal frente às suas tentativas de se adaptar ao ambiente em que se encontra (BROOM, 1986). Portanto, quanto maior o desafio imposto pelo ambiente, mais dificuldade o animal terá em se adaptar e, consequentemente, menor será seu grau de bem-estar (BROOM e MOLENTO, 2004).

Para avaliar o bem-estar dos animais de criação, é necessário desenvolver e utilizar métodos de aferição baseados na resposta dos animais. Nos procedimentos de diagnóstico centrados no animal, os indicadores mais utilizados são as respostas fisiológicas e comportamentais e a sua condição sanitária (LEEB et al., 2004). Esses indicadores proporcionam uma avaliação mais precisa do bem-estar, pois fornecem informações diretas sobre a resposta e os efeitos sobre o animal. As medidas baseadas nas respostas animais são consideradas pela EFSA (Autoridade Europeia para a Segurança Alimentar), como os indicadores mais adequados para avaliar o bem-estar animal (EFSA, 2012). Em 2011, o projeto AWIN (Indicadores de bem-estar animal) foi fundado com o objetivo de melhorar o bem-estar de cavalos, ovelhas, macacos e perus, criando, integrando e disseminando informações sobre os indicadores de bem-estar animal. O projeto AWIN foi desenvolvido visando refinar os protocolos de avaliação usando indicadores animais, incluindo dor, nas espécies acima mencionadas, e tem como principal fundamento os quatro princípios de bem-estar (ausência de fome e sede, conforto, boa saúde, poder expressar seu comportamento natural), e mais vinte critérios desenvolvidos pela Welfare Quality e descritos na tabela 1, sendo estes completos, mas não complexos, de maneira que possam ser aplicados para as necessidades atuais. O resultado dos protocolos visa fornecer um feedback visual claro e imediato aos pecuaristas sobre o bem-estar dos animais na fazenda, destacando as condições positivas e permitindo a comparação com uma população de referência. Os protocolos AWIN foram criados para permitir comparações entre sistemas de produção e gestão semelhantes e destinam-se a avaliar o bem-estar dos animais, a fim de orientar a sua melhoria na Europa e em outras partes do mundo.

TABELA 1 – PROTOCOLO DE AVALIAÇÃO DE BEM-ESTAR PARA CAVALOS

Princípios de Bem-Estar	Critério de Bem-estar	Indicadores de Bem-Estar
Boa Alimentação	Nutrição Apropriada	Escore Corporal
	Ausência de sede prolongada	Disponibilidade de água
Bom Alojamento	Conforto para descansar	Cama
		Dimensões das Baias
	Conforto térmico	Não considerado para cavalos alojados individualmente
	Facilidade de movimentação	Exercício
Boa Saúde	Ausência de injúrias	Alterações na pele
		Joelhos inchados
		Laminite
		Prolápso
	Ausência de doenças	Condição da pelagem
		Corrimentos
		Consistência das Fezes
		Respiração anormal
		Tosse
	Ausência de dor e procedimentos de manejo dolorosos	Horse Grimance Scale
		Sinais de negligência dos cascos
		Lesões na boca e comissuras
Comportamento Apropriado	Expressão de comportamento social	Interação social
	Expressão de outros comportamentos	Estereotipias
		Teste do medo
	Boa Relação com o ser-humano	Testes de relacionamento humano-animal
	Estado emocional positivo	Avaliação Qualitativa do Comportamento

FONTE: ADAPTADO DE AWIN WELFARE ASSESSMENT PROTOCOL FOR HORSES (2015).

Espécies como ovelhas, cabras, cavalos, macacos e perus são comumente menos estudadas, e por isso ainda há pouca informação disponível. Sendo assim, há constante demanda por estudos que possam criar e validar novos indicadores de bem-estar animal dessas espécies (AWIN, 2015).

2.2.2 Práticas de manejo que afetem o bem-estar do cavalo atleta

A criação de animais da espécie equina possui grande importância para o Brasil, onde são utilizados para esporte, trabalho, lazer e como terapia para pessoas com deficiência ou com necessidades especiais. O crescimento destes usos fez com que os cavalos que viviam predominantemente em áreas rurais passassem a viver também em áreas urbanas (VIEIRA, 2015). Atualmente, a maioria dos criadores de cavalos busca um ambiente prático, seguro e limpo, fazendo com que muitos estabelecimentos optem por manter seus animais estabulados, sem contato suficiente com outros cavalos, e muitas vezes privados de interagir com o ambiente (MILLS e CLARKE, 2002). Cavalos de alto desempenho atlético tendem a passarem a maior parte de seu tempo estabulados, podendo assim desenvolver mais facilmente injúrias e desordens fisiológicas que afetam seu nível de bem-estar. O estresse crônico está diretamente relacionado com a restrição alimentar, dietas com altos níveis de concentrado e teor insuficiente de fibras, dificuldade para exercer comportamentos espécie-específicos do animal, além da restrição locomotora e o isolamento social (McBRIDE e HEMMINGS, 2009). A impossibilidade de expressar o seu comportamento natural gera grandes períodos de ócio, o que pode levar ao surgimento de comportamentos indesejados, sendo assim um indicador de baixo grau de bem-estar (MILLS E NANKERVIS, 2005).

Outro estímulo estressante na rotina de cavalos atletas além do alojamento em baias, é o treinamento físico constante. Durante um exercício extenuante, o cavalo pode precisar aumentar a sua taxa metabólica em até 60 vezes com o objetivo de prover o transporte de oxigênio, água, eletrólitos, nutrientes e hormônios para os músculos em contração, além de retirar da circulação substâncias como o dióxido de carbono e outros metabólitos produzidos durante o esforço físico (MCCUTCHEON e

GEOR, 2008). Esta sobrecarga fisiológica leva ao distúrbio da homeostase corporal, fazendo do exercício físico um estímulo estressor (MCKEEVER, 2002).

Entretanto, os protocolos de treinamento diferem quanto à intensidade do exercício físico. Alguns estudos realizados em seres humanos afirmam que o exercício físico moderado pode diminuir os níveis de ansiedade, depressão e raiva, considerados sintomas de estresse, e também pode reduzir a influência de fatores de estresse psicossociais sobre o indivíduo (CREWS e LANDERS, 1987; BERGER e MACINMAN,1993). O exercício tem se mostrado tão efetivo quanto as técnicas mais tradicionais na redução dos níveis de estresse em seres humanos, com a vantagem de evitar o uso de drogas (BAHRKE e MORGAN, 1978; LONG, 1985; LONG; HANEY, 1988; BERGER e MACINMAN, 1993). De acordo com pesquisadores da área de saúde humana, o bem-estar psicológico e físico pode variar de acordo com o tipo de exercício realizado e com os fatores envolvidos na prática, como o ambiente, os instrutores e o próprio indivíduo (SAMULSKI e LUSTOSA, 1996). Segundo WARING (2003), a prática regular de exercícios físicos também traz benefícios para a saúde fisiológica e psicológica do cavalo, sendo capaz de manter e melhorar o condicionamento físico. O treinamento seria capaz também de aliviar o tédio, trazendo diferentes estímulos ao animal, além de fornecer uma via para a liberação do excesso de energia acumulada em um cavalo estabulado, podendo assim impedir o aparecimento ou a ocorrência de comportamentos anormais. Sendo então desejável a implantação de um programa com atividades de trabalho variadas, objetivando motivar o cavalo e gerar melhor condicionamento físico.

Entretanto, os cavalos atletas de alto desempenho possuem uma rotina de treinamento constante e intensa, que quando associada ao manejo inadequado, pode gerar estresse crônico nos animais (ALEXANDER e IRVINE, 1998; LEAL, 2007). As alterações neuroendócrinas e hormonais causadas pelo exercício podem mediar alterações na função das células imunes e, portanto, influenciar a susceptibilidade à doença. Pesquisas realizadas com seres-humanos demonstraram que competições e treinamentos extenuantes têm sido relacionados a aumento aparente na susceptibilidade de atletas altamente treinados a infecções (NIEMAN, 1988). Ainda não se tem muitos estudos sobre qual das categorias de trabalho poderia trazer melhores índices de bem-estar para os cavalos. Sendo assim, o presente estudo se faz importante para a determinação do nível de exercício capaz de trazer melhorias no bem-estar e saúde de equinos mantidos integralmente em regime de confinamento em baias durante o treinamento para corridas.

2.3 MENSURAÇÃO DOS NÍVEIS DE ESTRESSE

O estresse fisiológico é um dos principais indicadores usados na avaliação do bem-estar animal. Estresse pode, de maneira geral, ser considerado a resposta fisiológica do organismo a um estímulo do ambiente, na tentativa de manter a homeostasia (HOTZEL E MACHADO FILHO, 2000). Nesse sentido, o estresse tem valor regulatório. Mas quando o estresse é prolongado, ou crônico, por meio da ação continuada de catecolaminas e glicocorticóides, tem repercussões negativas no sistema imunológico, reprodutivo e no crescimento (MENDL et al., 2001).

Sempre que existe estresse negativo, há diminuição do grau de bem-estar. Acredita-se que um animal tem seu bem-estar comprometido quando ocorre falência de adaptação ou quando o indivíduo está encontrando dificuldades em se ajustar (MOSTL E PALME, 2002; BROOM E MOLENTO, 2004). O córtex e a medula adrenal são os principais participantes na fisiologia do estresse, liberando cortisol e adrenalina, hormônios que aumentam a produção e o desvio de glicose para o sistema nervoso central e musculatura (GENUTH, 2000). A adrenalina também induz a mobilização de leucócitos do compartimento vascular marginal para o compartimento circulante, inicialmente aumentando o número de linfócitos, seguido de aumento nos números de neutrófilos e monócitos, resultando em leucocitose fisiológica, uma das alterações do sistema imunológico mais comumente relatadas decorrente do exercício intenso (BENSCHOP e RODRIGUEZ-FEUERHAHN, 1996). Após o término do exercício, há a fase de recuperação com a liberação de corticosteroides, especialmente o cortisol. Esse hormônio produz um efeito imunossupressor e anti-inflamatório, induzindo a neutrofilia pela diminuição rápida do número de linfócitos, e queda mais lenta dos neutrófilos, causando a alteração da relação entre os neutrófilos e linfócitos, o que tem sido relatado em muitos estudos (SNOW et al., 1983, BENSCHOP e RODRIGUEZ-FEUERHAHN, 1996).

Os parâmetros hematológicos são ferramentas valiosas comumente utilizadas para a avaliação da saúde e bem-estar dos animais. Os números dos leucócitos totais e a contagem diferencial de células são técnicas que permitem observar mudanças nos níveis circulantes de leucócitos associadas a alterações nas concentrações de catecolaminas e cortisol. Os mecanismos responsáveis pelas mudanças nos números e funções dos leucócitos em resposta ao exercício são complexos. No entanto, os hormônios neuroendócrinos desempenham um papel fundamental na modulação da resposta imune ao exercício. O aumento nas concentrações plasmáticas de catecolaminas e cortisol é influenciado pela intensidade e duração do exercício, além do nível de treinamento do indivíduo. Respostas hormonais em cavalos atletas são geralmente consistentes com aquelas observadas em seres humanos (MACNEIL et al., 1991). O consenso geral é que os aumentos das concentrações de catecolaminas plasmáticas causam o aumento da contagem de leucócitos durante o exercício, enquanto que o aumento da concentração do cortisol no plasma pode modular a leucocitose durante a recuperação (MACKINNON,1992).

Um estudo realizado com pôneis demonstrou que um programa de treinamento composto por cinco dias de exercícios extenuantes foi capaz de induzir a imunossupressão, aumentando da susceptibilidade dos animais ao vírus influenza (FOLSOM et al., 2001). Resultados semelhantes também foram encontrados em estudos realizados na área de saúde humana, demonstrando que o excesso de treinamento pode ter prolongado efeito imunossupressor, com aumento da susceptibilidade a doenças infecciosas, em particular, do trato respiratório superior (NIEMAN et al., 1990; SMITH, 2003). Entretanto, outros pesquisadores sugerem que o treinamento a longo-prazo com realização de atividade física moderada está associado com o fortalecimento da resposta imune e maior resistência à doença em humanos (KLENTROU et al., 2002). LOWDER, PADGETT e WOODS (2005) realizaram um experimento com camundongos e observaram que o exercício moderado, realizado nos primeiros dias logo após a infecção pelo vírus influenza, diminuiu significativamente a mortalidade desses animais. No entanto, o exercício prolongado levou ao aumento da morbidade e tendeu a diminuir a taxa de sobrevivência.

Apesar dos aumentos nas concentrações de hormônios neuroendócrinos terem sido medidos em associação com as alterações da função imunitária em várias espécies, uma relação direta ainda não foi estabelecida em cavalos e necessita ser melhor investigada por meio de novas pesquisas.

Outra variável utilizada para avaliar estresse e bem-estar animal é a incidência de comportamentos anormais. Comportamentos anormais são considerados um redirecionamento de comportamentos para os quais o animal tem forte motivação, mas cuja realização está impedida por fatores ambientais. A ocorrência e a frequência de comportamentos anormais podem ser usadas para avaliar a adaptação do animal a um ambiente de cativeiro (HOTZEL E MACHADO FILHO, 2000).

O comportamento é uma consequência da interação entre os fatores genéticos e o meio ambiente, sendo que classificação de um certo comportamento como anormal depende da duração, frequência e motivação (MILLS E NANKERVIS 2005; GONTIJO, 2010). Dentre os comportamentos anormais, as estereotipias são os problemas comportamentais mais discutidos e preocupantes em cavalos. Segundo MILLS e NANKERVIS (2005), comportamentos estereotipados podem ser definidos como "comportamentos repetitivos, relativamente invariáveis e sem função aparente". Contudo essa definição pode ser considerada muito subjetiva (MASON, 2006). Comportamentos estereotipados têm sido vistos em uma variedade de espécies tais como suínos, macacos, bovinos e girafas; entretanto são dificilmente observados em animais que nunca estiveram em cativeiro (MILLS e NANKERVIS, 2005).

As formas mais comuns de estereotipias em equinos podem ser divididas em duas categorias gerais; oral e locomotoras. Estereotipias locomotoras podem estar relacionadas ao confinamento e à carência de contato social, sendo ambos problemas associados com a estabulação (WARAN, 2001). Alguns exemplos de estereotipias locomotoras são:

- "Dança do urso": Ato de balançar a cabeça e o pescoço, podendo às vezes alterar o peso entre as patas dianteiras (WARAN, 2001).

- Andar em círculos na baia: O animal pode caminhar em círculos dentro da baia por horas a fio, em um padrão repetitivo (HOUPT e McDONNELL, 1993).

- Meneio de cabeça: Movimento no qual o cavalo balança verticalmente a cabeça e o pescoço (COOPER et al., 2000).

- Escavar e chutar: Estereotipia constituída por ações como escavação excessiva com as patas, chutar paredes e portas. Pode estar presente em momentos estimulantes, como antes da entrega da alimentação (HOUPT e McDONNELL, 1993).

Estereotipias orais podem ser associadas com a restrição alimentar ou erros de manejo (WARAN, 2001). Alguns exemplos de estereotipias orais são:

- Mastigar madeira: Ato de morder a madeira com os dentes incisivos, podendo às vezes ocorrer a ingestão desse material. (MILLS e NANKERVIS, 2005).

- Lamber excessivamente: Essa estereotipia é caracterizada pela lambedura excessiva de superfícies como cochos e paredes (LEWIS, 2000).

- Aerofagia sem apoio: Comportamento onde o cavalo parece engolir ar repetidamente, podendo ser acompanhado por um grunhido (MILLS e NANKERVIS, 2005).

- Aerofagia com apoio: Consiste no ato de morder com os dentes incisivos uma superfície quase sempre horizontal, enquanto contrai a musculatura ventral do pescoço, envolvendo ou não o ato de engolir ar (MCGREEVY et al., 1995b).

A prevalência de estereotipias em cavalos estabulados em diversos estudos variou entre 5 e 25% da população analisada (MCDONNEL, 2002). Em um trabalho de NICOL (1999), as frequências encontradas foram: 8,3% para aerofagia de apoio, 9,5% para dança do urso e 7,3% para andar em círculos na baia. Em um estudo a prevalência de estereotipias em uma população de cavalos com uso para adestramento, concurso completo de equitação e enduro foram 32,5%, 30,8% e 19,5% respectivamente (MCGREEVY et al., 1995a). Já em um trabalho realizado em centros equestres no Brasil, com diferentes usos do cavalo a prevalência de comportamentos anormais variou de 21% a 95% entre os grupos (LEME et al., 2014).

Sendo assim, o número de animais executando comportamentos anormais ou estereotipias em um determinado ambiente pode constituir um bom indicador de redução no seu grau de bem-estar (BROOM E JOHNSON, 1993). Entretanto, apesar de esse tipo de avaliação ser capaz de detectar alterações no comportamento, não deve ser interpretada sem outros parâmetros, pois fatores como características individuais do animal, espécie, raça, experiência prévia do animal e o tipo de agente estressor podem influenciar no comportamento animal o que pode causar falha na interpretação e gerar resultados subjetivos (CLARK, RANGER E CALPIN, 1997; MOSTL e PALME, 2002).

Atualmente os métodos mais utilizados e mais aceitos como avaliação do estresse em cavalos, consistem na mensuração do cortisol, análise dos parâmetros hematológicoas e na análise da resposta comportamental. Entretanto esses métodos possuem também grandes limitações. A avaliação do cortisol plasmático requer um procedimento invasivo de coleta de sangue, o que pode consistir de um estímulo estressante e potencialmente confundir os resultados. A amostragem fecal para cortisol não consiste um método adequado para a avaliação do estresse agudo, uma vez que reflete um nível médio de cortisol ao longo do tempo, em vez da amostragem pontual oferecida pelo plasma. Além disso, a análise laboratorial para a avaliação do cortisol é demorada e dispendiosa (YARNELL, 2011).

A avaliação comportamental é um método prático, que não requer a utilização de equipamentos específicos e está disponível para ser utilizada por pessoas comuns que convivem com cavalos em seu dia-a-dia, como proprietários e tratadores. Entretanto, essa técnica pode ser considerada subjetiva e, como o cavalo é uma espécie que na natureza é vista como presa, os equinos podem mascarar sinais de estresse como mecanismo de sobrevivência. Isso significa que a resposta comportamental pode não ser uma medida confiável de bem-estar, quando analisada separadamente. Devido a estas limitações, não existe atualmente uma medida única, instantânea, confiável e não invasiva para a mensuração da resposta ao estresse para o cavalo (YARNELL, 2011).

Quando um animal é exposto ao estresse ou dor, a preparação de uma resposta é inicialmente desempenhada pela divisão simpática do sistema nervoso autônomo, com posterior estímulo da medula adrenal que secreta as catecolaminas (adrenalina e noradrenalina). Estes hormônios afetam vários sistemas e órgãos que preparam o organismo para uma resposta de "luta ou fuga". As pupilas dilatam-se para aumentar a acuidade visual e o fluxo sanguíneo é redistribuído para o esqueleto e músculos cardíacos. A constrição dos vasos sanguíneos da pele protege contra perda excessiva de sangue em caso de danos aos tecidos externos e há o aumento do fluxo sanguíneo nos órgãos internos e nos músculos (STEWART et al., 2005). A constrição dos vasos sanguíneos em órgãos externos resulta na redução da emissividade térmica desses locais (NAKAYAMA et al., 2005) e por isso, as alterações associadas a temperatura da superfície corporal podem ser medidas de forma não invasiva usando termografia infravermelha (MOURA et al., 2011), podendo fornecer uma indicação objetiva e mais imediata da resposta ao estresse quando comparadas à avaliação comportamental e à análise hormonal.

A mais nova geração de câmeras de termografia infravermelha realiza medições em tempo real. O equipamento é leve/portátil e exibe grande sensibilidade. Além disso, o software da câmera permite a análise de dados de temperatura em qualquer área do termograma. O método de termografia infravermelha tem muitas aplicações, particularmente na indústria, medicina e ciência veterinária (GODYN at al., 2013).

3 CONSIDERAÇÕES FINAIS

O surgimento de criações de cavalos situadas em ambientes urbanos tem se tornado cada vez mais comum. Esse tipo de propriedade muitas vezes possui espaço físico restrito, onde não há possibilidade de soltar todos os animais. Sendo assim, o aumento da intensidade do exercício físico poderia ser recomendado como uma ferramenta para a diminuição do nível de estresse dos animais confinados e contribuir com a melhoria do seu grau de bem-estar. Para avaliar qual intensidade de treinamento de corrida proporciona melhor grau de bem-estar para cavalos Puro-sangue-inglês, é necessário desenvolver e utilizar métodos de aferição baseados na resposta dos animais. Amostras de sangue podem ser facilmente coletadas de animais que estão acostumados a serem manejados. A técnica de contagem diferencial de células pode ser realizada a baixo custo e ser utilizada para o monitoramento do estresse de animais durante o treinamento. A termografia infravermelha e, especificamente, a medição da temperatura máxima ocular, tem se destacado como uma ferramenta não invasiva que pode ser utilizada para medir estresse agudo e crônico em outras espécies, mostrando um grande potencial. Esses métodos de avaliação, juntamente com a análise comportamental, podem trazer uma ampla visão sobre o atual nível de bem-estar dos animais de corrida em treinamento. Entretanto, os resultados de pesquisas utilizando animais da espécie equina ainda são controversos e essas tecnologias necessitam ser melhor estudadas em cavalos. Ainda há relatos de grande variação nas respostas ao exercício físico, tornando difícil a realização de recomendações específicas. Entender melhor a influência de diferentes níveis de exercício sobre indicadores de bem-estar animal, pode fornecer ferramentas para melhorar a qualidade de vida de equinos atletas, bem como melhorar o seu desempenho e vida útil, uma vez que o estresse crônico pode trazer diversos males ao organismo desses animais. Sendo assim, se faz necessária a realização de estudos que objetivem avaliar o bem-estar de equinos mantidos em regime de confinamento em baias, quando submetidos à diferentes intensidades de exercício durante o treinamento para corridas.

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CAPÍTULO I - PREVALENCE OF ABNORMAL BEHAVIORS IN THOROUGHBRED HORSES STARTING THE RACE TRAINING

Summary

The stress generated by the confinement in stalls and consequently, the incapacity to express the natural behavior is one of the main causes of behavioral disorders in horses. It has already been consolidated that physical activity is able to reduce stress and anxiety in humans, so the increase of physical exercise could be an alternative to improve the horse's well-being, especially in establishments that have restrict physical space, like Jockey Clubs. Therefore, the aim of this trial was to use the prevalence of abnormal behaviors as a tool to evaluate the welfare of racehorses submitted to an increasing level of exercise intensity. This study used 16 thoroughbred horses housed in individual stalls at Paraná's Jockey Club, in Curitiba, Brazil. The horses were exercised 5 days per week for an average time of 20 minutes during a period of 5 months, which has been divided into 3 increasing levels of intensity. Direct focal observations were made during 1 day in each training level every 10 minutes during the period of 10 hours. The behaviors were analyzed through descriptive statistic and using the data mining technique with the application of algorithm J48. The software used for the analysis was Weka®. Two classes were selected in order to show more contrast: the second and third exercise intensities against the first exercise intensity and two decision trees were built. The first tree was generated without feature selection and found that bed-eating, attacking the air and box kicking were more frequent in the first exercise intensity, whereas wood-chewing was more often seen in the morning during the first exercise intensity and in the afternoon during the second and third exercise intensities. The second decision tree was generated using the feature selection and it emphasizes the relation of bed-eating to the low level of physical activity. The model found no more differences in the others stereotypies and abnormal behaviors during the three different levels of exercise intensity, although could be assumed that high level of exercise intensity had no negatives influences on the behavioral responses of stabled horses as well.

Keywords: welfare, stereotypies, stabled, stress, abnormal behaviors, exercise intensity, data mining

1 INTRODUCTION

Horses are herbivores and gregarious animals that in their natural environment, can travel long distances searching for food and spending an average time of 16 hours per day grazing (ZANINE et al., 2006). However, with the advents of the domestication, the role of the horse in human history has changed across time. When the horses were first domesticated about 6,000 years ago, they were maintained as a food source (LEVINE, 1999), but nowadays they have become high performance athletes, taking a fundamental part in the practice of equestrian sports. The growth of this use brought the need to breed and to keep these animals also in urban areas, contributing to increase the number of establishments that choose to keep the animals stabled (GOODWIN, 2007). However, it must be emphasized that confinement is incompatible with the horse's natural behavior and the imposition of this type of management can decrease the welfare of horses, causing psychic, physical and metabolic disorders that will detriment the animal's welfare. The stress generated by the confinement in stalls can be considered the main cause of the appearance of behavioral disorders in horses (GONTIJO, 2010).

The diet of elite performance racehorses consists of feeding high quality concentrates and relatively low amounts of forage and many times they don't have access to pasture. The low frequency of the meals offered and the consequently food deprivation (SARRAFCHI, 2012), combined with lack of social contact and restrict locomotion create long periods of idleness. The stress and boredom garnered by the incapacity of expressing the natural behaviors may lead horses to practice abnormal behaviors and stereotypies as a way to relieve frustration (HENDERSON and WARAN, 2001). Abnormal behaviors are not natural in free-ranging feral horses and the stereotypies are a category of abnormal behaviors that can be described as random, repetitive and seemingly non-functional behaviors (MASON, 1991; PESSOA et al., 2016).

If the time that a horse spend stabled can increase the risk of developing abnormal behaviors, changing the housing conditions could be a solution to improve the welfare of horses (VISSERA, ELLISB and VAN REENENA, 2008). However, equine centers located inside urbanized areas, like Jockey Clubs don't have enough physical space available, making impossible to release all the horses in open areas. In those situations, the increase of physical exercise could be an alternative to improve the animal's wellbeing. In the field of human health, it has already been consolidated that physical activity is able to improve mood and to reduce symptoms of depression and anxiety (ROSS and HAYES, 1988; STEPHENS, 1988). Another research made with Breton and Percheron horses housed in individual stalls found evidences that horses practicing physical exercise may present less stereotypies than sedentary horses (REZENDE et al., 2006). But it must be reminded that excessive physical exercise is also a source of stress, therefore more studies are needed to clarified the effect of different exercise intensities on horse well-being. So, the aim of this study was to study and evaluate the incidence of abnormal behaviors as an indicator of the welfare level, from the moment the animals started their race training, until the horses have been considered ready for their first official race.

2 MATERIALS AND METHODS

2.1 STUDY POPULATION

All experimental procedures were approved by the Local Ethical Committee for Experiments on Animals of the Universidade Federal do Paraná (UFPR, protocol number 033/2017). This trial used 10 mares and 6 stallions of thoroughbred horses housed in individual stalls, going out only to exercise, with no free time outdoors.

The study was carried out at Paraná's Jockey Club, in Curitiba, Brazil. The horses were about 2 years old and had an average weight of 500 Kg. They shared the same management protocol and the feeding routine was composed by three meals per day of alfalfa hay and a mix of concentrate ration and oat.

2.2 RACE TRAINING

The horses were exercised 5 days per week for an average time of 20 minutes during a period of 5 months, which has been divided into 4 increasing levels of intensity (treatments) as described in table 1.

TABLE 1 – DESCRIPTION OF THE TRAINING INTENSITIES APPLIED TO THE HORSES SINCE THE BEGINNING OF THE RACE TRAINING, UNTIL HAVE BEEN CONSIDERED READY FOR THEIR FIRST OFFICIAL RACE (FIVE MONTHS)

Treatments	Exercise Intensity	Duration	Description
First	Light	Two months	2000 meters of trot and 1000 meters of canter
Second	Moderated	Two months	500 meters of trot and 2000 meters of gallop
Third	Intense	One month	900 meters of trot and 1000 meters of fast gallop

SOURCE: The author

2.3 DATA COLLECTION METHOD

Direct focal observations were made during 1 day in each training level, using an interval sampling technique where the behavior of each horse was observed by six trained observers and instantaneity noted every 10 minutes during the period of 10 hours, totalizing 180 observations of each horse during the entire experiment (MATIN and BATESON). Observations were carried out at the stables on Sundays, when the horses are not exercised and are kept 24 hours in individual stalls. Each behavior observed was defined according to the ethogram (Table 2).

TABLE 2 - ETHOGRAM OF THE OBSERVED BEHAVIORS

Normal Behaviors	
Eating hay	Animal feeding on forage in troughs
Eating concentrate	Animal feeding on ration
Drinking	Animal seeking water or drinking water
Standing alert	Animal standing alert in place, aware with elevated neck, observing the surroundings.
Standing passive	Animal standing in place a little unaware or sleepy, observing the surroundings, with either head or ears turned at little time fractions.
Vocalizing	Sounds made by the animal.
Other natural Behaviors	Not listed above (defecating, lying down, urinating, scratching, walking, rolling over, yawning)
Stereotypies	
wood-chewing	The horse chews and tries to pull out wood pieces to eat them
Metal-chewing	The horse chews metal objects like iron grids
Excessive licking (OWEN, 1982)	To lick an object or surface without nutritional purpose
Box kicking	Knocks with the hind limb hoofs against the wall of the stable.
Nodding	Repetitive movements from the head up and down.
Pawing	The horse using the hoof for digging or scratching a surface
Box-walking	The horse wanders in circles inside the box, walking, trotting, or galloping.
Rearing	When a horse "stands up" on its hind legs with the forelegs off the ground
Attacking the air	Horse alone in the stall showing aggressive behavior
Other Abnormal Behaviors	
Bed-eating	The horse ingests the bed material of its stable
Coprophagy	To eat the own feces

SOURCE: adapted from ALTMANN (1974).

2.4 STUDY DESIGN AND DATA ANALYSES

The experiment was performed under a total randomized design with 16 replications. The behaviors were analyzed through descriptive statistic and using the data mining technique with the application of algorithm J48. The software used for the analysis was Weka® 3-4 (Witten & Frank, 2016). Two classes were selected in order to show more contrast: the second and third exercise intensities against the first exercise intensity.

The first phase included establishing the objective and then converting it into a Data Mining problem. The aim of this project was to determine if the different classes (Light and moderated Exercise Intensity against Intense Exercise Intensity) would influence the animals' behavior. The next phase, called data understanding, involved a literature review and initial data collection. Subsequently, the data preparation phase took place with the tabulation of data, data selection and selection of attributes in order to construct the final dataset (data that was fed into the modeling tool). The data base for the analysis included 21 attributes as listed on table 3. In the next phase, the modeling tool was run on the prepared dataset to create one or more models. The first tree was built without using feature selection and class balance, generating a more complex model. And the second tree was generated with a feature selection, used to remove the attributes with low correlation values, applying Weka's selection algorithm CfsSubsetEval. All generated decision trees were compared for accuracy rate, complexity based on the number of rules generated, and ability to understand these rules according to expert opinion (CHAPMAN et al., 2000). The experts were researchers and professors in animal science. The knowledge acquired from the decision tree can be represented by IF-THEN Rules for Classification. Each classification rule is a path from the root node to a leaf (a predicted class).

TABLE 3 – SUMMARY OF USED DATA AND FEATURES ASSUMED FOR ORGANIZING THE FINAL DATA SET.

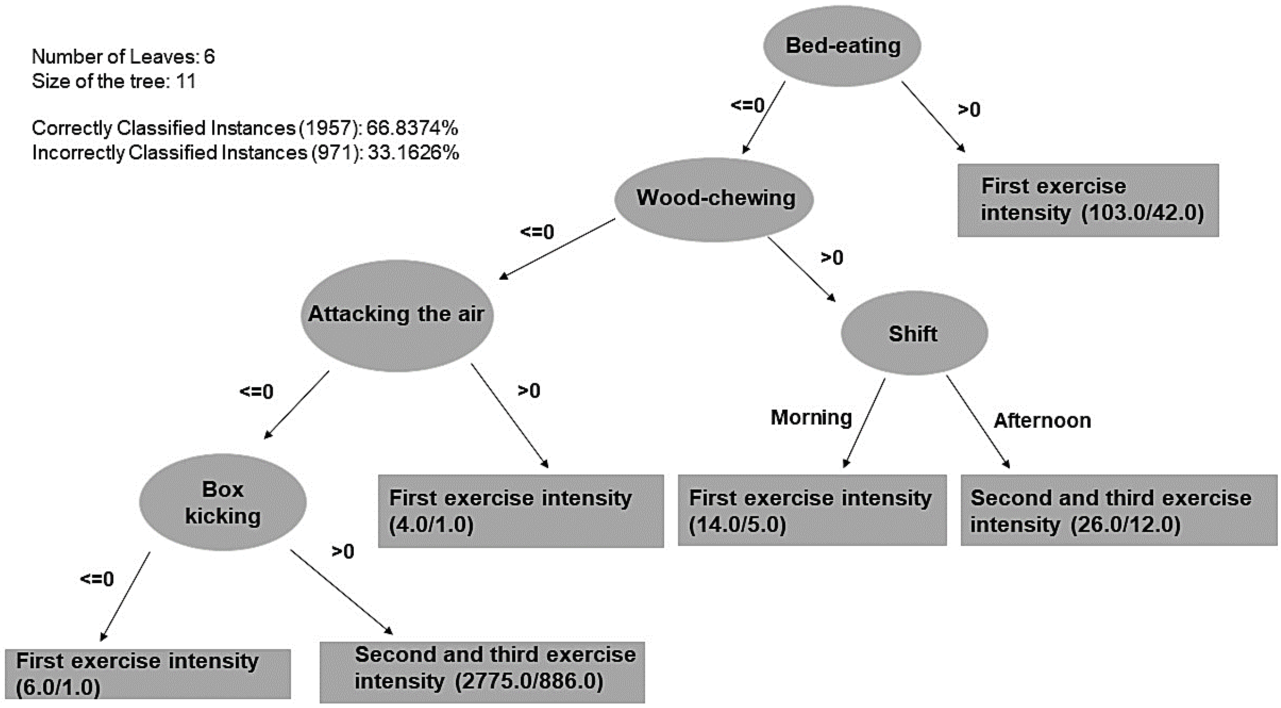
Nº	Feature	Nº	Feature
1	Feeding Time	12	Box-walking
2	Bed-eating	13	Standing alert
3	Coprophagy	14	Standing passive
4	Excessive licking	15	lying down
5	Metal-chewing	16	Walking
6	wood-chewing	17	Eating concentrate
7	Box-kicking	18	Eating hay
8	Rearing	19	defecating
9	Nodding	20	urinating
10	attacking the air	21	Drinking
11	Pawing		

SOURCE: the author

3 RESULTS

The decision tree generated from the model without feature selection and class balance is depicted in Figure 1. According to this model, bed-eating, attacking the air and box kicking were more frequent in the first exercise intensity, whereas wood-chewing was more often seen in the morning during the first exercise intensity and in the afternoon during the second and third exercise intensities.

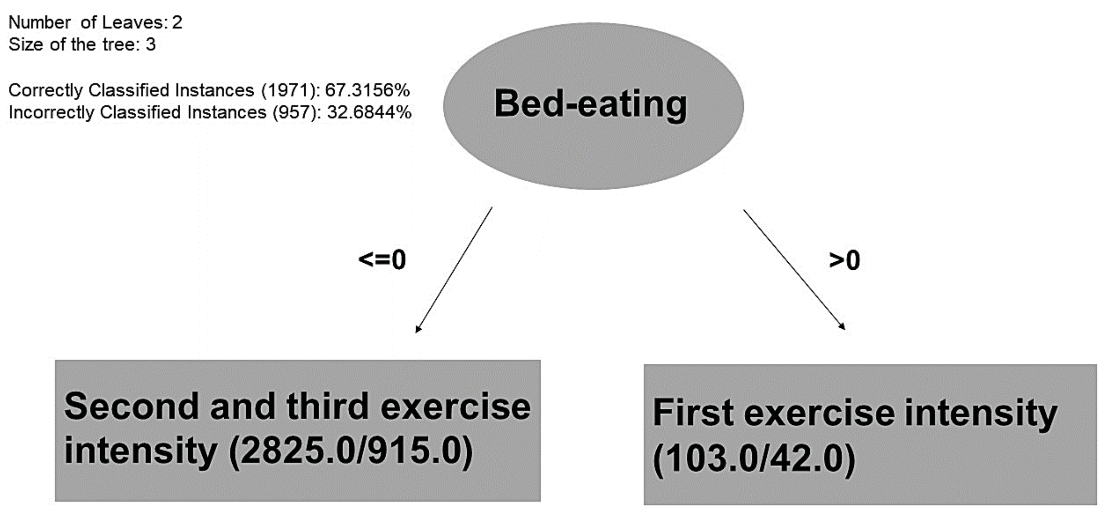
FIGURE 1 - DECISION TREE GENERATED FROM THE DATA WITHOUT FEATURE SELECTION (<=0 or >0 refer to the number of observed behaviors. Model accuracy = 66.84%).



SOURCE: the author

The decision tree generated using the feature selection to reduce the complexity of the model had similar accuracy to that one obtained without applying feature selection and can be seen in the figure 2. The second tree generated by the model emphasizes the relation of bed-eating to the low level of physical activity.

FIGURE 2 - DECISION TREE BUILT USING THE FEATURE SELECTION (<=0 or >0 refer to the number of observed behaviors. Model accuracy = 67.32%).



SOURCE: the author

The model found no more differences in the others stereotypies and other abnormal behaviors during the three different levels of exercise intensity. Although the data mining has shown no more results, a small decrease in the prevalence of stereotypies and other abnormal behaviors can be noticed through the nonparametric analyses (table 4). During the total duration of this trial, 100% of the horses have been observed bed-eating, and 93.75% of the animals have presented at least one kind of stereotyped behavior. Bed-eating and wood-chewing were the most often observed abnormal behaviors during the experiment. During the first exercise intensity, the horses have been observed practicing bed-eating for an average time of 18.44 minutes and wood-chewing for an average time of 5.62 minutes. During the third exercise intensity, these average times have decreased to 6.25 and 0.31 minutes respectively. During the first level of exercise intensity, 68.75% of the horses have practiced at least one stereotyped behavior and repeated these behaviors during an average time of 14.38 minutes, withal, 93.75% of the horses were observed practicing other abnormal behaviors (coprophagy or bed-eating), and they repeated these behaviors during an average time of 19.70 minutes. During the second level of exercise intensity, 43.75% of the animals were observed practicing coprophagy or bed-eating, spending an average time of 6.88 minutes on these behaviors, whilst 81.25% of the animals were practicing at least one stereotyped behavior and spending an average time of 12.81 minutes on them. The values for the third level of exercise intensity were 43.75% and 8.13 minutes spent practicing coprophagy or bed-eating, and 68.75% and 8.44 minutes spent for at least one stereotyped behavior. The number of animals practicing abnormal and stereotyped behavior has decreased and stabilized until the end of the training, as well as the time the animals spent repeating the behaviors. The natural behaviors more often observed were standing alert and standing passive. The time that the horses spent in those positions has increased with the training progression from an average of 77.5 and 48,13 minutes to 94.38 and 70 minutes respectively, in the meanwhile, the time the animals spent laying down have decreased from an average of 28.5 minutes to 15.84 minutes.

TABLE 4 - PERCENTAGE OF HORSES PRACTICING STEREOTYPIES AND OTHERS ABNORMAL BEHAVIORS WHEN SUBMITTED TO DIFFERENT INTENSITIES OF RACE TRAINING AND KEPT UNDER TOTAL CONFINEMENT.

Observed stereotypies and other abnormal behaviors	Exercise intensity			During the total training period
	First	Second	Third	
Wood-chewing	50.00	31.25	6.25	62.50
Metal-chewing	12.50	18.75	6.25	25.00
Excessive licking	43.75	31.25	37.50	87.50
Box kicking	6.25	6.25	0.00	12.25
Box-walking	12.50	12.50	6.25	31.25
Digging	18.75	25.00	12.50	50.00
Shaking the head	6.25	6.25	0.00	12.50
Attacking the air	6.25	0.00	6.25	6.25
Rearing	6.25	6.25	0.00	12.50
Ingesting the bed	93.75	37.50	43.75	100.00
Coprophagy	12.25	18.75	18.75	43.75

SOURCE: the author.

4 DISCUSSION

Studies about the prevalence of behavioral disorders in horses have revealed inconsistent results. The prevalence rates of 93.75% for stereotypies and 100% for other abnormal behaviors are higher compared with some previous studies using thoroughbred horses. TADICH et al. (2012) obtained a prevalence rate of 3.32% for stereotypies and 5.52 % for other abnormal behaviors, while REDBO et al. (1998) obtained a prevalence of 9.3% and 16.3% for stereotypies and others abnormal behaviors, respectably. BACHMANN and STAUFFACHER (2003) found 16,5% of horses in Switzerland, PRINCE (1987) found 1-4% of English Thoroughbreds. Those data seem to support the theory that behavioral disorders are closely associated to environmental factors (diet, bedding, exercise, social contact, management) and horse-specific factors (age, sex and breed). This dependence on multiple factors could explain the difficulty in finding a significant decrease in certain stereotyped behaviors.

The percentage of abnormal behaviors found in this trial are more similar to the rate of 95% found in horses used in rodeo. Wind-sucking and weaving were related to be the most frequent abnormal behaviors in rodeo horses, however, these behaviors were not observed during this trial (LEME et al., 2014). Some researchers believe that wind-sucking and crib-biting are stereotypies that may take a longer time to be learned and wood-chewing could precede or be associated with the development of crib-biting (NICOL, 1999). Bed-eating is another concerning kind of abnormal behavior that was observed all animals during the experiment and could potentially cause health issues. This behavior involves the chewing and ingestion of bedding substrates such as shavings (MILLS, ECKLEY and COOPER, 2016), it is more common in horses that do not have access to high fiber diets and it is often accompanied by nosing and sifting for forage that has fallen to the floor or larger pieces of wood. The animals seem to do it in order to balance the low fiber concentrated meal with more fibrous material or because of the perseverance of feeding behavior that cannot be satisfied by a small meal. Depending on the material composition of the bed, this habit could lead to chronic irritation of the respiratory epithelium from dust, noxious gases and fungal spores what may not only be unpleasant but can also lead to serious health problems (MILLS, ECKLEY and COOPER, 2016). According to the data mining analyses, the increase in the exercise intensity was able to reduce the incidence of this abnormal behavior, as well as box kicking and aggressive behaviors, such as attacking the air. These findings support the theory that exercise activity may be able to improve the wellbeing of animals. KRZAK, GONYOU and LAWRENCE (1991) conducted an experiment with nine yearling Quarter Horses housed in individual stalls and submitted to different exercise protocols. They concluded that wood-chewing occurs primarily during the late night and morning and that exercise reduces the time that stabled horses spend chewing wood. Although the increasing levels of physical exercise were not able to significantly reduce the prevalence of wood-chewing in the current study, there was a change in the habit, that used to be more common in the morning during the light training intensities and became more often seen in the afternoon during the moderate and heavy exercise intensities. This change may have been influenced by the fact that the training sessions were performed in the morning, even though that the behavioral observations were taken during the horses' day of rest. So, can be conclude that high exercise intensities had no negative influence on the behavioral responses of stabled horses and was able to decrease the prevalence of some abnormal behaviors. The behavioral assessments are a simple method that does not require specific equipment and can be used by ordinary people that are used to horses, like owners and grooms, to access the welfare level of horses.

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CAPÍTULO II – CHANGES IN BLOOD NEUTROPHIL/LYMPHOCYTE RATIO AND TOTAL LEUKOCYTES RELATED TO DIFFERENT EXERCISE INTENSITIES IN THOROUGHBRED HORSES

Summary

With the beginning of the exercise, the hypothalamic pituitary-adrenal axis is activated, raising the plasma concentrations of catecholamines and cortisol. These hormones play an important role mediating the body responses to the exercise, including changes in the immunity, affecting the total leukocyte counts as well as the neutrophils and lymphocytes ratio. Therefore, the goal of this trial was to use the white blood cell differential count to study the effects of increasing intensities of race training on the thoroughbreds' immunity and wellbeing. The study used 16 two-year-old thoroughbred horses housed in individual stalls at Paraná's Jockey Club, in Brazil. The horses were exercised 5 days per week for an average time of 20 minutes during a period of 5 months, which has been divided into 4 increasing levels of intensity. The first and the second level of exercise intensity lasted 2 months and were characterized respectively by: 2000 meters of trot and 1000 meters of canter, and 500 meters of trot and 2000 meters of gallop. The third level of exercise intensity lasted 1 month and was characterized by: 900 meters of trot and 1000 meters of fast gallop, and the horses' first official race was considered the highest exercise intensity. Blood samples were collected 24 hours after the highest physical effort of the animals at each stage of training and ANOVA was performed with post-hoc Tukey test. There was a significant increase in Neutrophil/Lymphocyte ratio during the intense exercise training and the first official race, and a significant increase in the total leukocytes number after the first official race. Although the different training intensities have significantly influenced some hematological parameters, all the results were enclosed in the normal physiological parameters. So, the animals were not being over trained and the exercise intensities of the training protocol did not cause suppression of the animals' immunity.

Keywords: immunity, differential leukocyte count, hematological parameters

1 INTRODUCTION

Homeostasis can be defined as the ability of an organism to keep your internal environment stable when dealing with external challenges (CANNON, 1929). Under resting conditions, the horse has a relatively easy job of maintaining the internal environment. However, the performance of work or exercise is a major physiological challenge. In order to transport oxygen, water, electrolytes, nutrients, and hormones to working muscles and also remove from the circulation carbon dioxide and other waste products produced during exercise, the horses increase their metabolic rate 40- to 60-fold higher during exercise (MCCUTCHEON and GEOR, 2008). This overload of the physiological processes leads to a disturbance of the homeostasis, making the physical exercise a stressor stimulus (MCKEEVER, 2002). The sympathetic nervous system (SNS) and the hypothalamic pituitary-adrenal axis are stress systems active during the exercise, raising the plasma concentrations of the catecholamines (epinephrine and norepinephrine) and cortisol (NAGADA et al, 1999). These hormones play an important role mediating the body responses to the exercise. The epinephrine and norepinephrine are derivatives of tyrosine produced by sympathetic nerve endings and the adrenal medulla (WEIHUA, 1993). They mediate a multitude of physiologic changes, exerting their effects within minutes of their induction and are considered to represent the active response to stress (CACIOPPO, 1998). Catecholamines mediate their effect through the interaction with adrenergic receptors found on the surface of various cells, including lymphocytes (LANDMANN,1992; CHAMBERS et al., 1993; MADDEN et al., 1995).

The exercise-induced leukocytosis is one of the most reported effects of exercise on the immune system seen after intense exercise and is characterized by an initial increase in lymphocyte numbers followed by an increase in neutrophil and monocyte numbers caused by the release of epinephrine (BENSCHOP and RODRIGUEZ-FEUERHAHN, 1996). Lymphocyte numbers have been shown to return to resting levels in as short as 1 h after exercise, whereas neutrophils are slower in returning to resting values and, considering the fact that lymphocyte numbers decline rapidly, are responsible for the increased neutrophil to lymphocyte ratio reported in many studies (SNOW et al., 1983; BENSCHOP and RODRIGUEZ-FEUERHAHN, 1996). Lymphocyte numbers have been shown to drop below baseline values in subjects during the recovery phase following intense exercise creating a lymphopenia, and this effect has been attributed to post-exercise production of cortisol (ROSSDALE et al., 1982). Based on these findings, a biphasic model of the immune response to the physical activity has been proposed: the acute stress would be responsible to enhances the immune response, whereas chronic stress would responsible for the suppression (SEGERSTROM and MILLER, 2004).

Since numbers of neutrophils and lymphocytes are affected by stress in opposite directions, researchers have often considered the relative proportion of neutrophils to lymphocytes an important tool able to measure the stress response, with this ratio being positively related to the magnitude of the stressor and proportional to the level of glucocorticoid release (DAVIS et al., 2008). Therefore, the goal of this trial was to use the white blood cell differential count to study the effects of increasing intensities of race training on the thoroughbreds' immunity and well-being, from the moment the animals started their race training, until the horses' first official race.

2 MATERIALS AND METHODS

2.1 STUDY POPULATION

All experimental procedures were approved by the Local Ethical Committee for Experiments on Animals of the Universidade Federal do Paraná (UFPR, protocol number 033/2017). This trial used 10 mares and 6 stallions of thoroughbred horses housed in individual stalls 24 hours per day, going out only to exercise, with no free time outdoors.

The study was carried out at Paraná's Jockey Club, in Curitiba, Brazil. The horses were about 2 years old and had an average weight of 500 Kg. They shared the same management protocol and the feeding routine was composed by three meals per day of alfalfa hay and a mix of concentrate ration and oat.

2.2 RACE TRAINING

The horses were exercised 5 days per week for an average time of 20 minutes during a period of 5 months, which has been divided into 4 increasing levels of intensity (treatments). The first treatment was constituted of race training with light exercise intensity. It lasted for two months, had 16 replications and can be characterized by: 2000 meters of trot and 1000 meters of canter. The second treatment was constituted of race training with moderated exercise intensity, had 16 replications, lasted 2 months and was characterized by 500 meters of trot and 2000 meters of gallop. The third treatment was constituted of race training with intense exercise intensity, lasted 1 month, had 16 replications and was characterized by: 900 meters of trot and 1000 meters of fast gallop. The horses' first official race was considered the fourth treatment, with the highest exercise intensity and had 10 replications. The description of each treatment can be seen in table 1.

TABLE 1 – DESCRIPTION OF THE TRAINING INTENSITIES APPLIED TO THE HORSES SINCE THE ARRIVAL OF THE ANIMALS TO THE JOCKEY CLUB, UNTIL THEIR FIRST OFFICIAL RACE (FIVE MONTHS)

Treatments	Exercise Intensity	Duration	Description
First	Light	Two months	2000 meters of trot and 1000 meters of canter
Second	Moderated	Two months	500 meters of trot and 2000 meters of gallop
Third	Intense	One month	900 meters of trot and 1000 meters of fast gallop
Fourth	Very intense	First official race	

SOURCE: The author

2.3 DATA COLLECTION METHOD

One blood sample was collected from the experimental animals in the last day of each treatment. Blood sampling was carried out at the stables in a resting day, when the horses are not exercised and are kept stabled in other to recover from the last exercise session and start a new exercise protocol in the next day. The blood samples were collected 24 hours after the last training and were drawn by jugular venepuncture into vacutainers with EDTA, in the first period of the morning, before brushing or feeding the animals. The blood samples were immediately stored at 4°C and transported to the Laboratory of Clinical Pathology of Universidade Federal do Paraná, where the leukogram with differential leukocyte count was determined. The values for the white blood cells (WBC) were determined by electron particle cell counter (BC-2800 Vet Auto Hematology Analyzer, Mingray). Blood smears were stained with a rapid Romanowsky-type stain (Newprov®, Brazil) for the determination of the differential leucocyte counts using a 100-cell counts technique. Differential cell counts were performed independently by 3 technicians counting 100 cells and the average of the 3 results was taken as the final reading. The neutrophil/lymphocyte ratio was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes (ROSSDALE et al., 1982).

2.4 STUDY DESIGN AND STATISTICAL ANALYSES

The experiment was performed under a total randomized design with 16 replications in the first, second and third treatments and 10 replications in the fourth treatment. The analysis of changes in total leukocyte, neutrophil, and lymphocyte counts was done with an ANOVA procedure with post-hoc Tukey test, and Bartlett's test was performed to verify the homogeneity of variances using the statistical program ASSISTAT, 2016 (The Assistat Software Version 7.7). The accepted level of significance was $P<0.01$.

3 RESULTS

There was a significant ($P<0.01$) increase in Neutrophil/Lymphocyte ratio during the intense exercise training and the first official race when compared to the other exercise intensities. A significant ($P<0.01$) increase in the total leukocytes and Neutrophils numbers could also be observed after the first official race. However, there was no difference between the light and moderate exercise intensities and the results for the absolute leukocyte counts and the percentage figures are shown in Table 2. The results indicate that the increase of the exercise intensity generates an increase in the Neutrophil/Lymphocyte ratio by increasing the number of total Neutrophils numbers and total leukocytes.

TABLE 2 – AVERAGE VALUES OF TOTAL LEUKOCYTES, NEUTROPHIL AND LYMPHOCYTES COUNT

Exercise Intensities	Leukocytes (ml)	Neutrophils (ml)	Lymphocytes (ml)	N/L Ratio
Reference Values	5,500-14,300	2,260-8,560	1,500-7,700	-
Light	8347±1356 **b**	4891±1143 **b**	3216±781 **ns**	1.603±0.540 **b**
Moderate	8767±1300 **b**	4923±853 **b**	3549±901 **ns**	1.465±0.421 **b**
Intense	8760±1686 **b**	5500±1446 **b**	2993±875 **ns**	1.988±0.834 **ab**
First race	11289±1528 **a**	7830±1229 **a**	3230±1261 **ns**	2.844±1.387 **a**

Numbers with different letters are significantly different (Tukey test) at 5% level. **ns** not significant (p> = .05). SOURCE: The author

4 DISCUSSION

The significant higher Neutrophil/Lymphocyte ratio found during the two highest exercise intensities in this trial is a consequence of an increase in the number of total Leukocytes, being the results compatible with what was founded in other studies in stressful situations. For example, MARIN et all. (2008) treated bulls during 49 days with a low daily dosage dexamethasone, which acted as endogenous cortisol, causing a decrease in the lymphocytes counts with an increase in neutrophil concentrations, and consequently, an increase of the Neutrophils/Lymphocytes ratio. ROSSDALE et al. (1982) demonstrated that an injection of adrenocorticotrophic hormone (ACTH) in thoroughbred horses was able to increase the plasma cortisol and generate significant alteration of the neutrophil/lymphocyte ratio 240 mins after the injection. They also reported similar results after 180 minutes of a gallop training, demonstrating the adrenocortical activity during exercise in adult horses. The exercise section was able to generate a significant increase in neutrophil count and a decrease in lymphocyte, altering the Neutrophil/lymphocyte ratio, which ranged from 1.5 to 2.5, what is very similar to the fluctuation between 1.5 and 2.8 found during the current study.

The significant increase in total leukocyte number found after the fourth treatment (first official race) could be attributed to the circulating epinephrine released into the blood stream at an early stage of exercise. This hormone plays a major role in leukocyte recruitment during exercise, causing the mobilization of marginalized cells from the peripheral vasculature or from the primary and secondary lymphoid organs (IVERSEN, 1994). In the meanwhile, the post-exercise production of cortisol is believed to be the main cause of lymphocyte numbers dropping below baseline values in human and equine subjects during the recovery phase following intense exercise, although this effect has not been observed in the present study, since a significant alteration in the lymphocyte counts has not been observed (WALSH et al., 2011; HOROHOV, 2008). All the blood samples analyzed during this trial had results enclosed in the normal absolute hematological parameters, even during the highest exercise intensities. Most studies that explore the relationship between physical activity and the immune system in horses evaluate the results after a single bout of exercise, making difficult to compare with the current study, where the horses have been accompanied during a long period of time and with different exercise intensities. The low total blood leukocyte counts is an abnormality that is often seen in overtrained humans athletes (SUZUKI et al., 1996; GLEESON, 2002). Researchers believe that prolonged exercise causes a large release of neutrophils from the bone marrow and repeated bouts of prolonged exercise over weeks or months could deplete the bone marrow of its reserves of mature neutrophils, making such individuals much more susceptible to infection. Although the horses have been exercised 5 days per week during a long period of time (5 months), the leukocyte counts were normal, indicating that the training protocol was not strenuous.

The mechanisms underlying excise-related changes in leukocyte subsets and their functions are multifactorial and can be related to the hormonal, metabolic and physiological changes that occur depending on the duration and intensity of the physical exercise. As all the results of the hemogram exams during the entire experiment were enclosed in the normal physiological parameters, could be concluded that, although the different training intensities have significantly influenced the leukogram, these changes were not enough to cause immune suppression. These results demonstrate that the animals were not being overtrained and the exercise intensities used in those training protocols did not cause suppression of the animals' immunity. Even though hemograms are not a usual part of the training routine in most part of the horse centers in Brazil, they can be used as a valuable tool to evaluate the welfare and also to prevent overtraining.

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CAPÍTULO III – EYE TEMPERATURE CHANGE ASSOCIATED WITH PHYSIOLOGICAL STRESS IN RESPONSE TO RACE TRAINING IN THOROUGHBRED HORSES

Summary

Within seconds of perceiving a stressor, two systems are activated: the sympathetic nervous system (SNS), followed by stimulation of the hypothalamic–pituitary-adrenal axis (HPA), resulting in an increase in catecholamines and cortisol concentrations. The release of these hormones alters the blood flow responses and, consequently, changes the heat production and heat loss from the animal that can be detected by the infrared thermography. So, the goal of this study was to observe and describe the eye temperature change associated with physiological stress in response to a session of race training in thoroughbred horses. Thermal images of the left eye of 13 thoroughbred horses were captured before and after the last training day before their first official race. The maximum eye temperature was used for the analyses, the respiratory rate before and after exercise was also measured and the ambient temperature and the relative humidity of the air at the stable and at the track were recorded. The experiment was performed under a total randomized design with 13 replications. The analysis of changes in maximal eye temperature was done with an ANOVA procedure and a correlation between the maximal eye temperature, respiratory rate and Comfort Index was investigated. There was a significant increase ($P<0.01$) in the eye temperature measurements taken after the training session. There was a positive linear correlation between the respiratory rate and the maximal eye temperatures. Therefore, the infrared thermography is a versatile, non-invasive and high sensitive technology that is able to effectively measure the eye temperature change, which consists in a useful parameter to measure stress levels in thoroughbred horses during race training.

Keywords: Infrared thermography, welfare, exercise, athletic horses

1 INTRODUCTION

In general, stress occurs when body homeostatic balance is disturbed. Therefore, exercise can be considered a stressor stimulus for which the body must find a new dynamic equilibrium. Prolonged or intense exercise cause system-wide alterations that necessitate integrated whole-body responses, requiring neural and endocrine mediation in order to maintain the internal environment within relatively narrow limits and provide an optimal condition for the cell function (McKEEVER, 2002). Within seconds of perceiving a stressor, two systems are activated: the sympathetic nervous system (SNS), followed by stimulation of the hypothalamic–pituitary-adrenal axis (HPA), preparing the horse for an immediate reaction that is termed the “fight or flight” response. Cortisol is produced by the adrenal cortex, whereas norepinephrine (noradrenaline) is synthesized by the medullary portion of the adrenal. Withal epinephrine (adrenaline) can be released from the sympathetic nerve endings by a local stimulus and also can be produced by the adrenal medulla. The increase in the catecholamines and cortisol enhance are responsible for preparing the horse for the intense physical activity involved during the flight response. These hormones induce the dilatation of the eyes, the increase in the rate and force of heart contractility, the constriction of blood vessels and the increase of blood pressure (PORGES, 1995). The blood is diverted from areas that are not considered important for the flight response including the gastrointestinal tract, reproductive, and immune system and taken to the sensory organs, skeletal muscle, lungs, heart and brain. The vasoconstriction of the skin may also be a protective mechanism to reduce blood loss in the case of injury (BLESSING, 2003).

Monitoring physiological responses, such as hypothalamic–pituitary–adrenocortical (HPA) activity and changes in plasma cortisol concentrations, in particular, are frequently used to study the stress responses in farm animals (BROOM and JOHNSON, 1993). However, is difficult to measure some physiological indicators of stress, like the hormones levels or the immunity activity because it requires blood sampling, which process itself can cause a stress response that may lead to confounding results. Besides, mostly of those techniques require highly specialized instruments and a long period of time and/or certain specific conditions for taking the sample from the animal (STEWART et al., 2005). Consequently, there is a need to develop an objective and non-invasive tool for stress assessment during equestrian competitions.

Previous studies have found that infrared thermography has a good potential as a non-invasive tool for stress assessment in horses during competitions (VALERA et al.; MCGREEVY, WARREN-SMITH AND GUISARD, 2012; BARTOLOMÉ et al., 2013). The release of the hormones during a stressful situation alters the blood flow responses and, consequently, changes the heat production and heat loss from the animal due to changes in peripheral blood flow (SCHAEFER et al., 2002), which can be detect by the infrared thermography. Specifically, the temperature of the eye and small areas around the posterior border of the eyelid and the *caruncula lacrimalis*, which have rich capillary beds innervated by the sympathetic system, respond to changes in blood flow. Some researchers have investigated changes in the eye temperature using the infrared thermography in horses. COOK et al. (2001) reported an increase in eye temperature and a significant correlation between maximum eye temperature and both salivary and plasma cortisol, suggesting that changes in eye temperature may be driven by activation of the hypothalamic–pituitary-adrenal axis. However, the specific mechanisms involved in the observed relationship between the hypothalamic–pituitary-adrenal axis and the infrared thermography responses are not known. Wherefore, the goal of this study was to observe and describe the eye temperature change associated with physiological stress in response to a session of race training in thoroughbred horses.

2 MATERIALS AND METHODS

2.1 STUDY POPULATION

All experimental procedures were approved by the Local Ethical Committee for Experiments on Animals of the Universidade Federal do Paraná (UFPR, protocol number 033/2017). This trial used eight mares and five stallions of thoroughbred horses housed in individual stalls at Paraná's Jockey Club, in Curitiba, Brazil. The horses were about 2 years old and weighing 460 ± 45 Kg. They shared the same management protocol and the feeding routine was composed of three meals per day of alfalfa hay and a mix of concentrate ration and oat.

2.2 RACE TRAINING

The horses have been exercised five days per week for an average time of 20 minutes during a period of five months. Thermal images from the left eye were captured before and after the last training day of the horses before their first official race. The training session at the experimental day lasted for 20 minutes and was characterized by 900 meters of trot and 1000 meters of fast gallop.

2.3 DATA COLLECTION METHOD

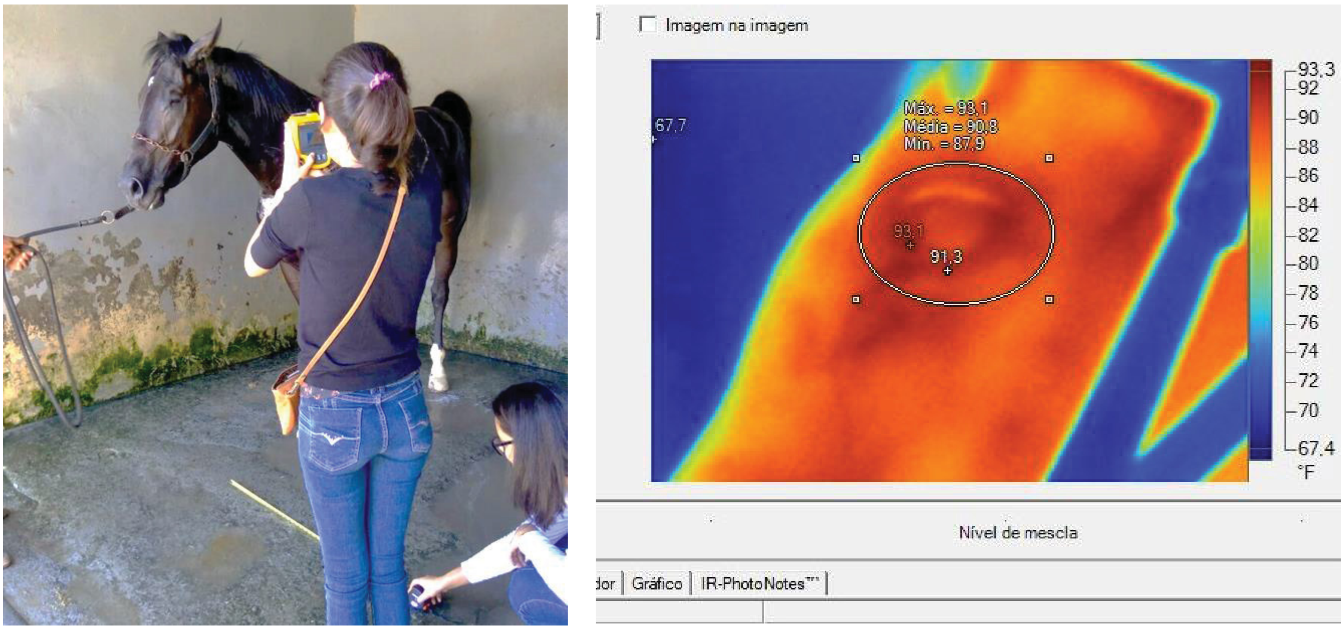
The temperature and the relative humidity of the air were recorded at five-minute intervals by two dataloggers, one placed at the stable and other placed at the track. A histogram was produced showing temperature and humidity over time and those data were used to assess environmental conditions by calculating the Comfort Index (CI) (JONES, 2009), as shown in equation 1:

$$CI = T_{air}\,(°F) + RH\,(\%) \tag{1}$$

The Comfort Index (CI) was used to monitor the potential effect of microclimate as a contributing factor to thermal change. The temperature and the relative humidity at the specific time of each thermal image were also selected in order to calibrate the camera results in the software during the images' analysis. Respiration rate was measured before and after exercise using a stethoscope.

One portable infrared thermal camera (Fluke Ti-25) was used to collect eye images. The images were collected two times: before the training session and after. Before the horses go to the track and after coming back to the stables, they were conducted one per time into the washing box, where the images were taken. The left eye of all horses was scanned from a 90º angle and at a distance of 1 m as shown in figure 1 a. Several images were taken per animal, selecting the image that provided the most optimal operating conditions for analysis (90º angleand 1 m of distance). Thermal camera software SmartView (Fluke Corporation, Everett, WA, USA) was used to measure the maximum temperature (°C) within an oval area traced around the eye, including the eyeball and about 1 cm around the outside of the eyelids (figure 1 b) (VALERA et al., 2012). The maximum eye temperature was used for the analyses.

FIGURE 1 - a: CAPTURING INFRARED THERMOGRAPHIC IMAGES OF THE LEFT EYE WITH THERMAL CAMERA. b: EXAMPLE OF THE IMAGES RECORDED FROM THE HORSES



SOURCE: The author (2017)

2.4 STUDY DESIGN AND STATISTICAL ANALYSES

The experiment was performed under a total randomized design with 13 replications. The analysis of changes in maximal eye temperature was done with an ANOVA procedure and Bartlett's test was performed to verify the homogeneity of variances using the statistical program ASSISTAT, 2016 (The Assistat Software Version 7.7). The accepted level of significance was $P<0.01$. The relationship between eye maximal temperature, Comfort Index (CI) and respiration rate was investigated using the simple linear correlation.

3 RESULTS

There was a significant difference ($P<0.01$) between the eye temperature measurements taken before and after the training session (table 1). The eye temperatures were significantly higher after the training. The eye temperature of all horses before exercise ranged from 33.56 C^o to 35.67 C^o, with the average value of

34.59 C°, whereas the temperature after exercise ranged from 34.94 C° to 37.56 C°, with the average value of 36.22 C°. The ambient temperature and the relative humidity of the air recorded at the stable and the track over the experiment duration can be seen in table 2. There was a significantly linear correlation between the maximal eye temperature and the respiratory rate, but correlations between the Comfort Index and maximum eye temperature or between the respiratory rate and the Comfort Index were not fond (table 3).

TABLE 1 – AVERAGE EYE TEMPERATURES (°C), RESPIRATORY RATE AND CONFORT INDEX OF THE HORSES BEFORE AND AFTER EXERCISE

	Before Exercise			After Exercise		
	Maximum Eye Temperature (°C)	Respiratory Rate (movements/ min)	Comfort Index	Maximum Eye Temperature (°C)	Respiratory Rate (movements/ min)	Comfort Index
Average Value	34.59±0.81	19.69±6.42	154.15±3.42	36.22±0.90**	55.08±19.61	155.35±2.35
Minimum Value	33.56	12.00	151.00	34.94	24.00	152.40
Maximum Value	35.67	32.00	159.50	37.56	92.00	159.50

** significant at the 1% probability level (p <.01), * significant at the 5% probability level (.01 = <p <.05)

SOURCE: The author (2017)

TABLE 2 – AVERAGE AMBIENT TEMPERATURE AND RELATIVE HUMIDITY OF THE AIR (RH%) AT THE STABLE AND TRACK

	Ambient Temperature Stable (°C)	RH Stable (%)	Ambient Temperature Track (°C)	RH Track (%)
Average Value	24.45±2.46	78.08±7.200	21.63±2.46	87.39±8.51
Minimum Value	20.70	68.10	18.60	69.20
Maximum Value	30.00	89.80	26.50	97.20

SOURCE: The author (2017)

TABLE 3 – COEFFICIENT OF CORRELATION CALCULATED BETWEEN MAXIMUM EYE TEMPERATURES AND RESPIRATORY RATE, MAXIMUM EYE TEMPERATURE AND COMFORT INDEX AND BETWEEN RESPIRATORY RATE AND COMFORT INDEX

Coefficient of Correlation	
Maximum Eye Temperature X Respiratory Rate	0.5909 **
Maximum Eye Temperature X Comfort Index	0.0979 **ns**
Respiratory Rate X Comfort Index	-0.0460 **ns**

** significant at the 1% probability level (p <.01), * significant at the 5% probability level (.01 = <p <.05), ns not significant (p> = .05)

4 DISCUSSION

Changes in the eye temperature related to stressful situations have been reported in several studies with cattle (STEWART et al., 2008) and horses (YARNELL, 2011; MCGREEVY, WARREN-SMITH and GUISARD, 2012; VALERA et al., 2012 BARTOLOMÉ et al., 2013;). YARNELL (2012) investigated the horses' physiological response to an actual potentially stressful husbandry procedure. She found a significant increase in eye temperature over the duration of the sham clipping treatment in all horses with the greatest effect of time at five minutes post onset of sham clip, suggesting that it may be driven by the sympathetic nervous system due to the rapid response at onset of the stressor. She also found a positive correlation between increase in eye temperature and increase in salivary cortisol during the sham clipping treatment, indicating that the horses found the procedure aversive and therefore the increase in eye temperature could suggest the same. Similar results were also found in a study evaluating the stress response of horses during show jumping competitions. VALERA et al. (2012) also found significant correlations between maximum eye temperature and salivary cortisol increases, concluding that both techniques are able to measure a physiological response to external stressors.

The scientific experiments executed using the infrared thermography to measure the eye temperature change occurring in response to stressful situations in horses have been founding relatively constant results. BARTOLOMÉ et al. (2013) found an increase in eye temperature in horses before and after a show jumping competition where the eye temperature ranged from 36°C to 38°C, whereas YARNELL (2011) found a range from 28.3°C to 37.5°C in the eye temperature of horses submitted to a sham clipping treatment and HALL et al. (2011) reported a range of 32.34 ± 2.61 °C after a lunging session. These results are consistent with what was found during the current study where the eye temperature ranged from 33.56°C to 37.56°C before and after a race training session.

Some experiments suggest that the environmental conditions could be a potential limiting factor in the use of the infrared thermography. CHURCH et al. (2014) have observed that environmental conditions like distance, wind speed, and solar loading can influence the eye temperature measurements. However, the same authors suggest that these effects could be minimized through the use of shelters or screens. Although the images of the current study have been collected during an entire morning with the ambient temperature variating from 20.70 to 30.00°C, a correlation between the Comfort Index and the maximal eye temperatures was not found. This result may be due to the fact that the animals were brought to the washing box during the image collection in order to protect them from wind or from direct exposure to the sun, keeping the maximal eye temperatures relatively constant while the ambient temperature was increasing along the morning.

According to JONES (2009), if the calculated value of the Comfort Index is less than 130, the horses are in the thermoneutral zone and the thermoregulatory system will not be activated. However, the calculated value of the Comfort Index during the images' collection before and after the exercise session were 154.15 ± 3.42 and 155.35 ± 2.35 respectively, characterizing thermal discomfort by heat.

In hot and humid conditions, the cutaneous evaporative cooling is compromised, increasing the proportion of the heat that is dissipated by the respiratory tract due to the elevation in the respiratory rate, which can represent 25% or more of total heat loss in these conditions. Although the Comfort Index has indicated heat stress, the horses' average respiratory rate observed before exercise was consistent with the normal respiratory rate of horses during rest (FEITOSA, 2004) and a correlation between the Comfort Index and the Respiratory Frequency has not been found. YARNELL (2011) studied the eye temperature of horses in every three hours over four consecutive days and no relationship between mean eye temperature and ambient temperature was found.

In another hand, the positive linear correlation found in the between the respiratory rate and the maximal eye temperature in the present study seems to reflect the release of catecholamines. These hormones help to prepare the animal for a “fight or flight” and are involved in the stimulation of respiration rate and also promotes the redistribution of blood flow during stressful situations. WILLIAMS et al. (2002) studied the neuroendocrine responses of horses to treadmill exercise on cool and hot humid environmental conditions and presumed that adrenaline and noradrenaline may play an important role in the adaptation of horses to thermal stress during training.

Therefore, can be concluded that the results obtained in this trial are consistent with those found in the literature. The infrared thermography is a versatile, non-invasive and high sensitive technology that is able to effectively measure the eye temperature change, which consists in a useful parameter to measure stress levels in thoroughbred horses during race training.

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CONCLUSÃO

Atualmente existem poucos estudos que investigam a relação dos esportes equestres com o bem-estar de cavalos. Esse fato deve-se principalmente a dificuldade de se realizar experimentos com a espécie, uma vez que os equinos atletas são animais de alto valor econômico, tornando difícil a aquisição e manutenção dos mesmos por instituições de ensino.

Em grandes centros equestres, como os Jockey Clubs, o manejo dos animais é feito de maneira dinâmica, praticamente industrial, tornando necessário o desenvolvimento de técnicas de avaliação do bem-estar rápidas, práticas e não invasivas. A rotina de cuidados e treinamento dos animais, aliadas ao temperamento energético da raça Puro-sangue inglês, fazem com que qualquer procedimento se torne bastante laborioso, sendo necessária a criação de métodos de avaliação do bem-estar animal cada vez mais simplificados.

A coleta de sangue com realização de hemograma completo e diferencial de células mostrou-se eficaz para monitorar a saúde e o bem-estar dos cavalos em treinamento, revelando que o exercício de alta intensidade não foi capaz de prejudicar os parâmetros hematológicos dos animais nem causou a supressão da imunidade. Os animais estavam acostumados ao manejo diário e ao contato com seres-humanos, fazendo com que a maior parte deles não reagisse negativamente às coletas. Entretanto, por se tratar de uma técnica invasiva, sua aceitação enfrentou grande resistência por parte dos tratadores e veterinários responsáveis, sendo impossível aumentar a frequência das coletas.

A análise comportamental dos animais é uma técnica não invasiva e que não sofreu muita resistência para a sua implantação. No entanto, ela necessitou de uma equipe grande com alunos previamente treinados, sendo difícil encontrar voluntários dispostos a ajudar na longa e cansativa coleta de dados. Os dados coletados também foram de difícil análise, fazendo com que essa técnica seja muito trabalhosa e demande muito tempo para ser realizada, o que também limitou o número de coletas. Outra questão limitante é que os animais precisam pertencer a uma mesma cocheira, pois o deslocamento até pavilhões diferentes é impossível devido ao curto intervalo de tempo entre as coletas. Após a primeira corrida oficial dos animais, não foi possível realizar a quarta e última coleta devido ao fato de que os animais foram separados e a maioria foi transportada para fora do Jockey Club.

A coleta de saliva para análise dos níveis de cortisol antes e após o exercício também enfrentou grande dificuldades e acabou se tornando inviável devido à falta de verba para a aquisição dos kits e equipamentos necessários para a análise das amostras. A coleta de saliva também se mostrou uma técnica de difícil execução, uma vez que os cavalos puro-sangue-inglês possuem um temperamento muito reativo, tornando muito complicadas as coletas realizadas principalmente após o exercício extenuante, havendo aumento da inquietação e, em alguns casos, menor presença de saliva. Algumas amostras de saliva também foram contaminadas por sangue, devido a ferimentos na boca dos animais acarretados pelo uso indevido das embocaduras. Sendo assim, pode-se concluir que a coleta de saliva não constitui um método adequado para a mensuração dos níveis de estresse gerados pela rotina de treinamento ou para competições equestres de cavalos da raça puro-sangue-inglês.

Já a utilização da câmera termográfica para a mensuração das mudanças na temperatura da superfície ocular se mostrou uma das ferramentas mais práticas e dinâmicas para medir os níveis de estresse agudo em decorrência do exercício físico. Apesar de não ser um método invasivo e não requerer equipamentos muito sofisticados para a análise dos dados, a câmera termográfica também possui grandes limitações que ficaram evidentes principalmente durante a primeira corrida oficial dos animais. Para que seja possível diminuir a influência dos fatores ambientais sobre os dados coletados, é preciso padronizar o local onde as imagens serão coletadas, o que se mostrou extremamente difícil durante uma competição equestre, principalmente após a corrida, onde os animais foram impedidos de entrarem nas baias para a coleta das imagens antes dos exames antidoping. Devido a essa restrição, algumas fotos foram tiradas a céu aberto e não puderam ser utilizadas na análise de dados. Outra limitação do modelo utilizado é a falta de resolução das imagens durante o período noturno, tornando muito difícil distinguir os animais através das imagens. Além disso, a câmera também possui pouca capacidade de registrar imagens de animais em movimento, o que tornou necessário coletar um grande número de imagens de cada cavalo, onde a maior parte dela saiu borrada, devido a inquietude característica da raça puro-sangue-inglês. Pode-se então concluir que a câmera termográfica constitui uma ferramenta promissora para a mensuração do estresse gerado durante o treinamento de cavalos atletas, dependendo apenas de melhores ajustes para servir adequadamente a essa função.

Sendo assim, conclui-se que o protocolo de treinamento com exercícios físicos de alta intensidade não gerou alterações negativas aos padrões hematológicos dos animais e foi capaz de diminuir a prevalência de alguns comportamentos anormais, o que parece ter sido benéfico, dentro dos parâmetros analisados. O exame de sangue com hemograma completo e diferencial de células se mostrou uma ferramenta indispensável para o monitoramento da saúde e bem-estar de cavalos atletas e deveria fazer parte da rotina de todos os centros de treinamentos de cavalos. A câmera termográfica também demonstrou um grande potencial para aferição dos níveis de estresse dos animais durante a pratica de exercício físico, sendo necessário apenas melhorar treinamento de profissionais da área de equideocultura para que colaborem com o processo de coleta do material, assegurando um ambiente padronizado, onde os animais possam ser fotografados, além do desenvolvimento de câmeras termográficas capazes de capturar imagens com baixos níveis de luminosidade e melhor resolução para objetos em movimento.

ANEXO 1 – CERTIFICADO DE APROVAÇÃO DO PROJETO PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

UFPR 100 Anos 1912 - 2012

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 033/2017, referente ao projeto **"DIFERENTES INTENSIDADES DE EXERCÍCIO PARA O BEM-ESTAR DE CAVALOS DE CORRIDA EM TREINAMENTO"**, sob a responsabilidade de **João Ricardo Dittrich** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em reunião de 05/05/2017.

Vigência do projeto	Junho/2017 até Dezembro/2017
Espécie/Linhagem	*Equus caballus* (equídeo) / PSI
Número de animais	17
Peso/Idade	500 kg / 2 anos
Sexo	Ambos (7 machos e 10 fêmeas)
Origem	Jockey Club em Curitiba, Paraná

CERTIFICATE

We certify that the protocol number 033/2017, regarding the project **"DIFFERENT EXERCISE INTENSITIES FOR THE WELFARE OF RACE HORSES IN TRAINING"** under **João Ricardo Dittrich** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 1 of invasiveness, in session of 05/05/2017.

Duration of the project	June/2017 until December/2017
Specie/Line	*Equus caballus* (equidae) / PSI
Number of animals	17
Wheight/Age	500 kg / 2 years
Sex	Both (7 males and 10 females)
Origin	Jockey Club in Curitiba, Paraná

Curitiba, 5 de maio de 2017.

Chayane da Rocha

Chayane da Rocha

Coordenadora CEUA-SCA