UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Veterinária
Programa de Pós-Graduação em Veterinária

Tese

Obesidade em equinos da raça Crioula

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Pelotas, 2022

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Tese apresentada ao Programa de Pós-Graduação em Veterinária da Faculdade de Veterinária da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências (área de concentração: Sanidade Animal).

Orientador: Carlos Eduardo Wayne Nogueira

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Data da Defesa: 20/12/2022

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Agradecimentos

Agradeço primeiramente à Deus por sempre guiar meu caminho da melhor forma possível.

Aos meus pais pela educação que me proporcionaram, por serem exemplo de vida, trajetória e caráter, e por me transmitirem todo o apoio necessário em todas as minhas escolhas. A vocês, todo o meu amor.

Ao restante da minha família que da mesma forma sempre me apoiaram de forma incondicional.

Ao meu orientador Professor Dr. Carlos Eduardo Wayne Nogueira pelo exemplo de pessoa e profissional, parceria, pelas oportunidades, por toda confiança depositada em mim e no meu trabalho e pelos conhecimentos transmitidos ao longo desses 8 anos de convivência.

À professora Bruna da Rosa Curcio também por todo conhecimento transmitido, pelas oportunidades e convivência durante todo o período.

Ao médico veterinário Leandro Américo Rafael, pelo exemplo de profissional que és, por toda paciência e conhecimentos transmitidos ao longo dos anos de convivência, e por último, mas não menos importante, pelas manias dentro do bloco cirúrgico, que hoje se tornaram minhas também. A ti, minha admiração.

A todos os colegas de pós graduação e graduandos do grupo ClinEq pelos momentos compartilhados, parceria e perrengues também.

As amigas que fiz durante minha estadia em Pelotas e que vou levar no coração para o resto da vida Vitória Müller, Camila Wendt, Bruna dos Santos Suñe Moraes, Alice Correa Santos, Carolina Brasil, Natália Ribeiro, Jemhally Dillenbürg, Margarida Aires, Morgana Borges, Luiza Gheno e em especial a Rafaela Souza que além de ser minha parceira de trabalho diário, por ser essa amiga presente e compartilhar muitos momentos bons e ser apoio nos ruins também. A todas vocês, minha gratidão.

Um agradecimento especial também as minhas "hermanas arigós", parceiras de trabalho, convivência, lamentações, e muitas risadas nos EUA Camila Wendt e

Margarida Aires. Muito obrigada pelos momentos e histórias que compartilhamos até aqui.

"É muito melhor lançar-se em busca de conquistas grandiosas, mesmo expondo-se ao fracasso, do que alinhar-se com os pobres de espírito, que nem gozam muito nem sofrem muito, porque vivem numa penumbra cinzenta, onde não conhecem nem vitória, nem derrota."
Theodore Roosevelt

Resumo

Com a crescente valorização da raça Crioula e as diferentes modalidades esportivas de alta exigência as quais a mesma participa, houve também um aumento das criações em sistemas intensivos, os quais disponibilizam maior aporte energético de nutrição, aumentando o índice de animais que apresentam sobrepeso e obesidade. Dessa forma, a presente tese tem como objetivo abordar o tema da obesidade na raça Crioula em dois períodos importantes da criação. O primeiro artigo aborda a avaliação imune humoral de éguas com sobrepeso gestantes em resposta a uma vacina comercial. Trinta éguas Crioulas gestantes foram separadas de acordo com escore de condição corporal (ECC) em éguas com sobrepeso (ECC≥7/9) e éguas controles (ECC = 5-6/9), ainda em cada grupo, os animais também foram separados em vacinadas e controles. As éguas foram vacinas contra o EHV-1 em duas doses com intervalo de 21 dias e coletadas mensalmente por cinco meses para avaliação de anticorpos. Ambos os grupos vacinados tiveram aumento de anticorpos séricos neutralizantes após a vacina, porém, após a segunda dose não foi observado aumento de anticorpos em nenhum dos grupos. Não foi observado diferença entre os grupos de animais vacinados com ECC≥7 e ECC 5-6 em nenhum momento, sendo constatado que o escore corporal em si não altera a resposta humoral durante o terço final de gestação. Assim, esse trabalho demonstrou que a obesidade não foi um fator que influenciou na resposta imune humoral de éguas gestantes. O artigo 2 teve como objetivo avaliar a condição corporal e perfil metabólico dos participantes finalistas da prova do Freio de ouro, separados por sexo. Esse estudo avaliou 84 animais, 43 fêmeas não-gestantes e 41 garanhões da raça Crioula, os quais foram realizadas as medidas morfométricas de ECC, escore de crista (CNS), avaliação de tamanho de gordura na crista e tamanho de gordura da base da cola, sendo que a partir dessas medidas foi realizada determinação de porcentagem de gordura corporal (%GC), índice de massa corporal (IMC), massa gorda (MG), massa gorda livre (MGL), relação circunferência torácica e altura (CT:AC) e relação circunferência de pescoço e altura (CP:AC). Desses 84 animais, 53 foram coletadas amostra de sangue para avaliação de triglicerídeos, colesterol, lipoproteína de baixa densidade (LDL), lipoproteína de alta densidade (HDL), lactato desidrogenase (LDH), cretina quinase (CK) e adiponectina. Com relação aos escores, 21% dos participantes foram classificados com sobrepeso (ECC 7/9), e 49% com CNS≥3. Foi encontrada maior deposição de gordura na base da cauda e no pescoço nas fêmeas. As fêmeas também apresentaram maior %GC, MG e relação CT:AC e menor CP:AC quando comparadas aos machos. Das variáveis metabólicas, as fêmeas apresentaram maiores concentrações de LDH, LDL, CK, e menor concentração de adiponectina que os machos. Não foi observado diferença nas medidas morfométricas dos participantes que passaram para o último dia de

competição dos que foram desclassificados. A partir desse estudo foi possível observar que apesar da prova do Freio de Ouro ser de alta exigência física e de preparo dos participantes, uma porcentagem dos participantes encontravam-se com sobrepeso e com aumento de deposição de gordura regional no pescoço, o que pode predispor ao desenvolvimento de alterações metabólicas. No entanto, não foi encontrada nenhuma alteração metabólica relacionada ao aumento de adiposidade. Além disso, as fêmeas acumulam mais gordura do que os machos.

Abstract

With the increase in value of the Crioulo breed and the different high-demand sports categories in which it participates, there was also an increase in intensive breeding systems, which provide a greater energy supply of nutrition, increasing the rate of animals that are overweight and obese. Thus, this thesis aims to address the issue of obesity in the Crioulo breed in two important periods of breeding. The first article addresses the humoral immune evaluation of pregnant overweight mares in response to a commercial vaccine. Thirty pregnant Crioulo mares were separated according to body condition score (BCS) into overweight mares (ECC≥7/9) and control mares (ECC = 5-6/9), yet in each group, animals were also separated into vaccinated and controls. The mares were vaccinated against EHV-1 in two doses with an interval of 21 days and collected monthly for five months for antibody evaluation. Both vaccinated groups had an increase in serum neutralizing antibodies after the vaccine, however, after the second dose, no increase in antibodies was observed in any of the groups. No difference was observed between the groups of animals vaccinated with BCS≥7 and BCS 5-6 at any time point, and it was found that the BCS does not alter the humoral response during the final third of pregnancy. Thus, this study demonstrated that obesity was not a factor that influenced the humoral immune response of pregnant mares. Article 2 aimed to evaluate the BCS and metabolic profile of the finalists of the Freio de Ouro competition, separated by gender. This study evaluated 84 Crioulo horses, 43 non-pregnant females and 41 stallions, from which morphometric measurements of BCS, crest neck score (CNS), evaluation of fat depth at the neck and fat in the base of the tail were performed, From these measures, the percentage of body fat (%BF), body mass index (BMI), fat mass (FM), free fat mass (FFM), thorax and height ratio (CT:AC) and neck to height ratio (CP:AC) were also performed. From the 84 animals, 53 were sampled for evaluation of triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), creatine kinase (CK), and adiponectin. Regarding BCS, 21% of the participants were classified as overweight (BCS 7/9), and 49% with CNS≥3. Greater fat deposition was found at the base of the tail and neck in females. Females also had higher %BF, FM and CT:AC ratio and lower CP:AC when compared to males. Of the metabolic variables, females had higher concentrations of LDH, LDL, CK, and lower concentrations of adiponectin than males. No difference was observed in the morphometric measurements of the participants who passed to the last day of competition from those who were disqualified. Based on this study, it was possible to observe that despite the fact that the Freio de Ouro competition is of high physical demand and preparation of the participants, a percentage of the participants were overweight and with increased deposition of regional fat in the neck, which may predispose to the development of metabolic

changes. However, no metabolic changes related to increased adiposity were found. In addition, females accumulated more fat than males.

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Lista de Abreviaturas e Siglas

BCS	Body condition score
BMI	Body mass index
BF	Body fat
CNS	Cresty neck score (escore de crista do pescoço)
CP:AC	Relação circunferência do pescoço e altura de cernelha
CT:AC	Relação circunferência torácica e altura de cernelha
CK	Creatina quinase
CO2	Dióxido de carbono
DICT50	50% tissue culture infective doses
ECC	Escore de condição corporal
EMS	Equine metabolic syndrome
EHV-1	Equine herpesvirus type 1
FBS	Fetal bovine serum
GC:HW	Ratio girth circumference and height at the withers
GC	Gordura corporal
HDL	High-density lipoprotein (lipoproteína de alta densidade)
IMC	Índice de massa corporal
LDL	Low-density lipoprotein (lipoproteína de baixa densidade)
LDH	Lactato desidrogenase

MEM	Meio essencial mínimo
MG	Massa gorda
MGL	Massa gorda livre
MHC	Major histocompatibility complex
NC:HW	Ratio neck circunference and height at the withers
PMN	Polymorphonuclear leucocyte
RK-13	Normal rabbit kidney epithelial cells
S.E.M	Standard error of the mean

Lista de Símbolos

≥	Maior ou igual
=	Igual
®	Registrado
R$	Reais
°C	Grau Celsius
º	Grau
+	Somatório
'	Minutos
''	Segundos
%	Porcentagem
µL	Microlitros
±	Mais ou menos
MG/DL	Miligramas por decilitro
KG	Quilogramas
IU	international units
CM	centímetros
U/L	unidades por litro

Sumário

1 Introdução

A raça Crioula descende de animais trazidos da península Ibérica, os quais após um longo período compondo manadas selvagens, se adaptaram a condições adversas de alimentação e temperaturas da América do Sul, e passaram a expressar características importantes como a rusticidade, e hoje, a raça encontra-se amplamente difundida nas Américas. Atualmente o Brasil possui um total de mais de 400 mil animais distribuídos em seu território, movimentando só no ano de 2017, mais de R$ 130 milhões (ABCCC, 2022).

A ascensão e a valorização do cavalo Crioulo, considerando como destaque as provas funcionais e exposições morfológicas, as quais avaliam desempenho físico e características físicas que definem a tipicidade da raça, implicou também em mudanças no padrão de criação e manejo nutricional destes animais. A alteração para uma dieta rica em energia com carboidratos de alto índice glicêmico, associada ao confinamento em cocheiras e por muitas vezes exercício controlado, resultaram no aparecimento de animais com sobrepeso e obesidade, condição a qual a raça Crioula é predisposta devido a sua descendência e adaptação. Como consequência da obesidade, evidenciamos o aparecimento de diversas outras condições que ameaçam o bem estar e sobrevida desses animais.

Atualmente, a associação de criadores de cavalos Crioulos (ABCCC) possui regulamentadas quatorze diferentes modalidades esportivas em que a raça participa (ABCCC, 2022), todas as quais possuem alta exigência física e metabólica. Dessas provas, a principal considerada como meio de seleção da raça é a prova do Freio de Ouro. Esta, é constituída por sete etapas, nas quais cada conjunto, cavalo e ginete, precisam obter uma pontuação, que ao final somadas indicarão os quatro melhores colocados. Para participação do Freio de Ouro, os animais passam por um longo período de treinamento físico e precisam ser classificados em provas que ocorrem durante o ano todo.

O sistema de criação de um campeão da raça inicia desde o cuidado das matrizes. Éguas matrizes da raça Crioula são geralmente mantidas em sistema extensivo de criação, com acesso livre a pastagens de alto valor nutritivo. Esses

animais, geralmente iniciam o período gestacional com alto peso corporal, facilitando o acúmulo de tecido adiposo ao longo da gestação. Vários trabalhos evidenciaram diversos problemas que a obesidade pode causar na égua gestante (OUSEY et al., 2008; GEORGE et al., 2011; BRYDEN, 2013; PEUGNET et al., 2016; ROBLES et al., 2017; ROBLES et al., 2018).

A obesidade é considerada uma condição patológica, caracterizada por acúmulo excessivo de tecido adiposo, e além de estar relacionada a uma série de alterações metabólicas, causa um estado de inflamação crônica e assim, tem sido também associada a disfunção do sistema imune. Dessa forma, os objetivos desse trabalho foram avaliar a imunidade humoral de éguas Crioulas gestantes com sobrepeso criadas sob um sistema extensivo. Além disso, em uma segunda etapa, foi realizada avaliação de escore corporal e perfil metabólico dos participantes finalistas de um ciclo do Freio de Ouro.

2 Artigos

2.1 Artigo 1

Humoral immunity is not altered in overweight pregnant Crioulo mares

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Full article

Humoral immunity is not altered in overweight pregnant Crioulo mares

Imunidade humoral não é alterada em éguas Crioulas gestantes com sobrepeso

Abstract

Both pregnancy and obesity can influence the occurrence of significant changes in the immune system; on this basis, the present study proposes to evaluate the humoral immune response of overweight pregnant mares in response to a commercial vaccine. Thirty pregnant Crioulo mares were separated according to body condition score (BCS) into overweight (BCS≥7/9) or lean-control (BCS= 5-6/9). In each group, the animals were subdivided into vaccinated and controls. The mares were vaccinated against EHV-1 in two doses spaced 21 days apart and had their blood collected monthly, for five months, for antibody evaluation. Both vaccinated groups had an increase in specific neutralizing antibodies after the vaccine; however, after the second dose, there was no increase in antibodies in any of the groups. Vaccinated overweight and lean-control mares did not differ at any time point. Therefore, this study demonstrated that obesity is not a factor influencing humoral immune response in pregnant Crioulo mares.

Keywords: vaccine, immune system, EHV-1, gestation.

Resumo

Tanto a gestação quanto a obesidade podem influenciar o desenvolvimento de alterações significativas no sistema imune, portanto, o presente estudo teve como objetivo avaliar a resposta imune humoral de éguas gestantes com sobrepeso em resposta a uma vacina comercial. Trinta éguas Crioulas gestantes foram separadas de acordo com o escore de condição corporal (ECC) em éguas com sobrepeso (ECC≥7/9) e éguas controles (ECC=5-6/9), e ainda em cada grupo, os animais também foram separados em vacinados e controles. As éguas foram vacinadas contra o EHV-1 em duas doses com intervalo de 21 dias, sendo realizadas coletas de sangue mensalmente durante cinco meses para avaliação de anticorpos neutralizantes. Ambos os grupos vacinados tiveram aumento de anticorpos neutralizantes específicos após a vacina, porém, após a segunda dose, não foi observado aumento de anticorpos em nenhum dos grupos. Nenhuma diferença foi observada entre éguas vacinadas com sobrepeso e as éguas controles em nenhum momento. Assim, este estudo demonstrou que a obesidade não é um fator que influencia a resposta imune humoral de éguas Crioulas gestantes.

Palavras-chave: vacina, sistema imune, EHV-1, gestação.

Introduction

Obesity is a complex problem in equine breeding that is related to several life-threatening conditions. It is commonly accompanied by metabolic alterations and dysfunctional secretion of adipokines and inflammatory cytokines, and may also be associated with the development of disturbances in immune responses (Knowles & Grieve, 2020; Salinas et al., 2020). Increased concentrations of inflammatory cytokines both in adipose tissue and blood

circulation have been described in obese horses (Johnson et al., 2009; Basinska et al., 2015). This inflammatory state is associated with chronic activation of the immune system, in which pro-inflammatory cytokines are constantly recruited and interfere negatively with immune function (Frasca & Blomberg, 2020).

Data from experiments with humans and mice suggest that obese individuals are at greater risk of contracting infections and that the response to vaccination is often impaired in these individuals (Milner & Beck, 2012). Compromised B cell function due to inflammation was suggested as a cause for failure to respond to vaccination (Frasca & Blomberg, 2020). Other immune components of innate and adaptative response are also implicated in the failure of antibody production following vaccination (Frasca & Blomberg, 2020; Ghanemi et al., 2021). In horses with metabolic alterations, a probable decrease in an adequate cellular response to vaccine challenge has been identified, without changes in humoral response (Elzinga et al., 2018). Other studies also demonstrated changes in the production of neutrophil reactive oxygen species in non-metabolically-altered obese horses and impaired peripheral blood polymorphonuclear phagocytosis in obese insulin-resistant horses (Salinas et al., 2020; Salinas et al., 2022).

Pregnancy also involves a complex relationship with the immune system in which the balance between the recognition of self and foreign antigens becomes essential for the development of a healthy fetus (Morelli et al, 2015; Antczak, 2020). During this period, the mother develops tolerance to the semi-allogeneic fetus through a series of interactions between the maternal immune system and the fetal-placental unit (Morelli et al, 2015; Antczak, 2020). The maintenance of the conceptus is possible through the modulation of an adaptive immune response throughout the gestational period (Fedorka et al., 2020).

In addition, the hypothesis that the maternal immune system would be suppressed throughout the gestation period to prevent the recognition and rejection of the fetus was mistakenly supported for a long time (Billingham et al., 2003; Mor et al., 2017). However, a responsive immune system is essential for the implantation and growth of the fetus (Mor et al., 2017). Thus, different immunological mechanisms, both innate and adaptive, are important for fertilization, implantation, and the maintenance of pregnancy (Mor et al., 2017; Fedorka et al., 2020).

Considering that pregnancy and obesity can contribute to the development of significant changes in the immune system, the interaction of both can affect maternal health and the fetus. The close relationship between obesity, pregnancy, and immune dysfunction is well-recognized in humans (Hersoung & Linneberg, 2002; Wilson & Messaoudi, 2015). Therefore, the present

study aimed to evaluate the humoral immune response of overweight pregnant mares in the final third of gestation after a vaccination.

Materials and Methods

Animals

All procedures performed in this study were approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (CEEA-UFPel) (approval no. 34960-2019). Thirty pregnant Crioulo mares aged between six and 11 years were included. The mares were to a farm in Santa Vitória do Palmar - RS, Brazil (33º31'08" S and 53º22'05" W), where they were housed exclusively in an extensive management system during the study.

The animals were selected based on pregnancy status and body condition score (BCS), which was assessed by the same trained observer at the beginning of data collection, based on the nine-point scale described by Henneke et al. (1983). The mares were divided into two groups: overweight (BCS≥7/9, n=20) and lean-control (BCS 5-6/9, n=10). Within these groups, the animals were subdivided between vaccinated (BCS≥7/9, n=13; BCS 5-6/9, n=6) and control (BCS≥7/9, n=7; BCS 5-6/9, n=4).

The mares were weighed on a digital electronic scale for large animals and evaluated for cresty neck score (CNS). The following morphometric measurements were also performed: neck circumference at 0.25, 0.50, and 0.75 portions along its length using a tape; crest neck height and tail head fat were obtained by ultrasonography (Sonoscape A5v, Domed dominium medical, São Paulo). All the above measurements were obtained as described before in Gentry et al. (2004) and Carter et al. (2009).

Blood samples and vaccine assay

Blood samples were obtained through venipuncture of the external jugular vein in grey-top tubes containing sodium fluoride and red-top tubes with no anticoagulant to assess baseline glucose and insulin, respectively. Both baseline insulin and glucose were evaluated at a single time point (300 days of gestation) as a control to identify whether any animal had increased concentrations. A commercial vaccine (Herpeshorse, Vencofarma®), provided by the manufacturer, was used to evaluate humoral immune response.

At about 300 days of gestation, blood was collected in red top tubes to measure neutralizing antibodies against equine alphaherpesvirus type 1 (EHV-1), and then, the first dose of the vaccine (2 mL) was administered. A booster dose was given at 321 days of gestation and

another blood sample was obtained to evaluate neutralizing antibodies. The control group received 2 mL of PBS+Al(OH)$_3$ at the same time points. After administration of the two doses, blood samples were obtained monthly for the following five months (until D155) to monitor antibody levels (Figure 1).

Glucose and insulin analysis

Baseline glucose was measured by the colorimetric enzymatic method, using commercial kits (Glucose Liquiform, Ref. 133) in an automatic analyzer (Labmax Plenno, Labtest Diagnóstica S.A., Minas Gerais, Brazil). The samples for basal insulin analysis were sent to the Pasin Laboratory - Clinical Analysis (Santa Maria, Rio Grande do Sul, Brazil).

Virus Neutralization Test

All serum samples collected in the study were tested for the presence of antibodies against EHV-1 using the virus neutralization assay as described elsewhere (OIE 2008). Briefly, 25 µL of MEM (minimum essential medium) with 10% FBS (fetal bovine serum) were distributed into 96-well microplates. Then, 25 µL of each sample were added and dilutions from 1:2 until 1:4096 were used. The viral suspension was prepared at a concentration of 100 DICT50 (50% tissue culture infective doses) and 25 µL were added to each well. The microplates were incubated at 37 ℃, in an incubator with 5% CO_2 for 1 h. Next, RK-13 cells at an approximate concentration of 3×10^4 were distributed to all wells and the plates were incubated again. The reading was done between 48-72 h. The titer of each sample was determined at the highest dilution in which no cytopathic effect was observed, and the cell layer remained intact.

Statistical analysis

Commercial software IBM SPSS Statistics 20 was used. Data normality was tested using the Shapiro-Wilk test. The variables not normally distributed were transformed to log10. Descriptive analysis of all morphometric measurements (weight, neck circumference at the three points, neck crest height, CNS, BCS, and tail head fat), glucose, and insulin are presented as mean ± SEM. A non-paired t-test was used for comparison between groups. Serum neutralization antibodies were analyzed by a repeated-measure ANOVA and are presented as log2. The difference was considered at $p < 0.05$.

Results

Weight, neck circumference at all points (0.25, 0.50, 0.75), crest neck height, and CNS were greater in the group with BCS ≥7 (Table 1). There was no difference in tail head fat between the groups. The group with BCS ≥7 had higher baseline glucose and insulin concentrations (74.81±1 mg/dL and 3.319 µIU/mL) than the group with BCS 5-6 (69.20±1.5 mg/dL and 1.190 µIU/mL), respectively.

All mares showed variable titers of serum-neutralizing antibodies against EHV-1 on the first day (D0). Three animals in the BCS ≥7 group had a baseline serum-neutralizing antibody titer ≥ 1024 and were excluded from the analyses. In the group with BCS ≥7, a difference was observed between vaccinated animals and controls at days 21, 35, and 95 after administration of the first dose (Figure 1). When evaluating the titers between days after the first dose of the vaccine, there was an increase in neutralizing antibodies from D0 to D35 in the group with BCS ≥7.

There was no difference between vaccinated animals and controls in the BCS 5-6 group; however, the curve of vaccinated animals increased from D0 to D155 (Figure 1). Among the vaccinated animals, there was no difference in neutralizing antibody response between the groups with BCS ≥7 and BCS 5-6 at any time point (Figure 3).

Discussion

The overweight and lean-control pregnant mares used in this study responded to vaccination by producing neutralizing antibodies against EHV-1. However, the second dose of the vaccine did not provide a boosting effect on antibody production. Despite this, humoral immune responses lasted up to 155 days post-vaccination.

Overweight mares showed higher values of weight, neck circumference, crest neck height, and CNS. Among all morphometric measurements evaluated, CNS has been considered more effective than BCS to differentiate animals with a greater predisposition to the development of metabolic alterations (Fitzgerald et al., 2019). Changes in glucose and insulin dynamics have been identified in animals with fat deposition above grade 2 (Carter et al., 2009; Fitzgerald et al., 2019). All overweight mares in this study had a CNS ≥2; however, although baseline glucose and insulin concentrations were higher in this group than in the group with BCS 5-6, they were within the reference range for the species (Frank et al., 2014).

The assessment of baseline insulin and glucose concentrations should be interpreted with care. Although insulin sensitivity can be evaluated using baseline measurements, this test is not considered the best choice for identifying animals with metabolic alterations (Frank, 2014; Dunbar et al., 2016). In these cases, dynamic tests are preferred (Dunbar et al., 2016);

however, we chose not to perform dynamic tests in this study so as not to interfere with the work routine at the property. Furthermore, besides the fact that no test was performed to identify insulin resistance secondary to obesity, it is important to consider that the mares were sampled in the final third of pregnancy, which is physiologically characterized by an increase in insulin resistance that occurs to increase glucose supply for the development of fetal-placental tissues (Fowden et al., 1984; George at al., 2011).

Both vaccinated groups had an increase in neutralizing antibodies after the first vaccination. Nonetheless, no seroconversion was observed after the second dose in either group. Bannai et al. (2019) found a similar effect after vaccinating non-pregnant animals with pre-existing antibodies, suggesting that animals that have already had contact with EHV do not have a boosting effect after a second dose. Another study that evaluated the effect of sequential vaccination in mares from an EHV-free area also did not observe an increase in antibodies after repeated vaccinations (Wagner et al., 2015). Likewise, antibody titers declined but were still present after the second dose until D155 in both vaccinated groups, which is described to occur after the use of inactivated vaccines against EHV-1 (Kydd et al, 2006; Bannai et al., 2019). The animals tested in this study had no previous history of vaccination; however, both control and vaccinated animals showed variable titers of neutralizing antibodies at all time points. These observations may suggest the occurrence of previous and/or recurrent contact of the animals with EHV-1, which usually happens in equine populations in field situations.

Pregnancy poses a constant challenge to the maternal immune system, and species that exhibit more invasive types of placentation, such as humans and rodents, had to develop several tolerance mechanisms to maintain the semi-allogeneic fetus (Antczak, 2020). The equine placenta is epitheliochorial diffuse, which is considered the least invasive among placental species (Meeusen et al., 2001). However, between days 25 and 36 of gestation, trophoblast cells assume an invasive profile and form endometrial cups (Meeusen et al., 2001; Antczak et al., 2013). After their formation, invasive endometrial cells express class I MHC of maternal and paternal origin, generating an immune response in which high titers of paternal anti-MHC I antibodies develop (Chen et al., 2012; Antczak et al., 2013; Antczak, 2020). This ability to develop antibodies suggests that humoral immune response is maintained and functions normally during pregnancy (Antczak, 2020), which was observed in this study. All pregnant mares responded to vaccine administration with increased antibodies after the first dose regardless of the group.

There was no difference in humoral response between the vaccinated overweight and lean-control groups. Similarly, Elzinga et al. (2018) evaluated the vaccine response in horses

presenting with equine metabolic syndrome (EMS) and found no effect of metabolic status on antibody response after vaccination. On the other hand, metabolic status in obese horses interfered with peripheral blood PMN phagocytosis (Salinas et al., 2022). Obesity was an important factor in humoral response and neutralizing capacity in a study conducted on mice (Kim et al., 2012). In that same study, obese individuals vaccinated against influenza and challenged with the H1N1 virus exhibited compromised immune response, increased inflammatory response, and increased mortality rate (Kim et al., 2012). In horses, obesity with no endocrine disorders was shown to alter neutrophil reactive oxygen species production (Salinas et al., 2020).

One limitation of the present study was the use of mares that already had prior antibodies against EHV-1. Although this is what is usually observed in field situations in equine populations on farms, it may have interfered with the assessment of immune response in both groups. In this respect, the use of seronegative animals would be more appropriate. Another factor to take into account is that we were not able to evaluate cellular immunity, which could be impaired in both gestation and in overweight animals. Further research should address cellular immunity in overweight pregnant mares.

Conclusion

Pregnant Crioulo mares with BCS 5-6 and BCS≥7 vaccinated against EHV-1 responded to vaccination by producing specific neutralizing antibodies. However, a second dose of the vaccine did not induce a boosting effect, and humoral immune responses lasted at least up to 155 days post-vaccination. Overweight pregnant mares showed no difference in humoral immune response to vaccination when compared with lean-control pregnant mares. This study demonstrated that obesity is not a factor that influenced humoral immune response in pregnant mares.

Conflict of Interest

The authors have no competing interests.

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Table 1. Morphometric measurements in pregnant lean-control (BCS 5-6/9) and overweight (BCS ≥7/9) mares. Data are presented as Mean ± S.E.M.

Morphometric measures	Lean-control mares (BCS 5-6/9)	Overweight mares (BCS≥7/9)
Weight (Kg)	455,44 ± 20,58[a]	583,11±9,16[b]
Neck circunference 0.25 (cm)	71,20±0,82[a]	74,81±0,81[b]
Neck circunference 0.50 (cm)	89,40±1,34[a]	95,26±1,14[b]
Neck circunference 0.75 (cm)	106,40±1,43[a]	119,29±1,42 [b]
Neck crest height (cm)	5,80±0,36[a]	8,48±0,41[b]
Tailhead fat (cm)	1,48±0,15	1,83±0,17
CNS	1,33±0,5[a]	2,57±0,5[b]
BCS	5,67±0,5[a]	7,67±0,48[b]

Different letters represent statistical difference between groups.

Figure 1- Timeline showing time points of vaccination and blood collection from mares starting at 300 days of gestation (D0).
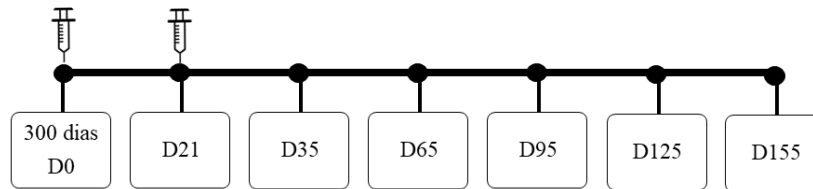
Figure 2- a) Serum-neutralizing antibodies in control and vaccinated mares with BCS 5-6 from D0 (baseline) to D155. **b)** Serum-neutralizing antibodies in vaccinated and control mares with BCS ≥7. The arrows indicate vaccination (first and second doses). Different letters indicate statistical difference between time points. Asterisk (*) indicates the difference between control and vaccinated groups at each time point, $p < 0.05$. The data represents mean±S.E.M of log2 transformed variables.
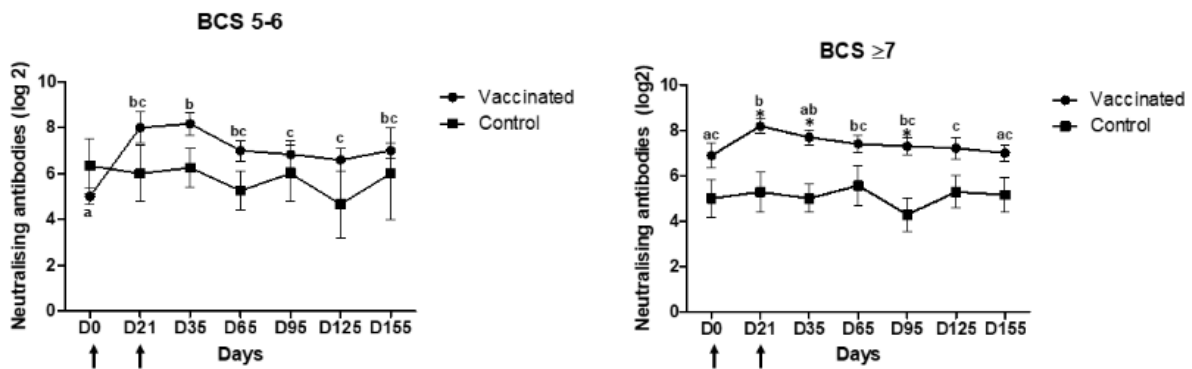
Figure 3- Serum neutralizing antibodies in vaccinated mares from groups BCS 5-6 and BCS ≥7, from D0 to D155. The arrows indicate vaccination. The data represents mean±S.E.M of log2 transformed variables.
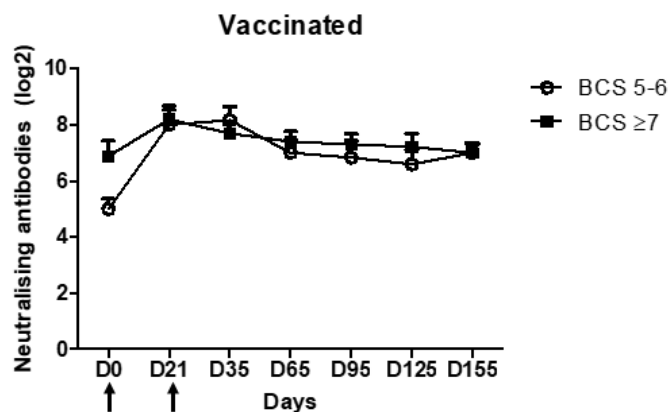
2.2 Artigo 2

Metabolic profile and body condition score of Crioulo horses finalists of the Freio de Ouro competition

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Metabolic profile and body condition score of Crioulo horses finalists of the Freio de Ouro competition

Perfil metabólico e escore de condição corporal de cavalos Crioulos finalistas da prova do Freio de Ouro.

Highlights

Twenty-one percent of the Freio de Ouro finalists were classified as overweight.

There were no metabolic changes related to increased adiposity.

Females were noticed to accumulate more fat than males.

Abstract

The present study aimed to evaluate the body condition score (BCS) and metabolic profile of the finalists of the Freio de Ouro competition, separated by gender. This study evaluated 84 Crioulo horses, 43 non-pregnant females and 41 stallions, from which morphometric measurements of BCS, crest neck score (CNS), neck and thorax circumference, evaluation of fat depth at the neck and fat in the base of the tail were performed, From these measures, the percentage of body fat (%BF), body mass index (BMI), fat mass (FM), free fat mass (FFM), thoracic circumference and height ratio (CT:AC) and neck circumference to height ratio (CP: AC) were also performed. From the 84 animals, 53 were sampled for evaluation of triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), creatine kinase (CK), and adiponectin. Regarding BCS, 21% of the participants were classified as overweight, and 49% with CNS≥3. The greater fat deposition was found at the base of the tail and neck in females. Females also had higher %BF, FM, and CT: AC ratio and lower CP:AC when compared to males. Of the metabolic variables, females had higher concentrations of LDH, LDL, CK, and lower concentrations of adiponectin than males. No difference was observed in the morphometric measurements of the participants who qualified for the last day of competition from those who were disqualified. Based on this study, it was possible to observe that even though the Freio de Ouro competition is of high physical demand and preparation of the participants, a percentage of the participants were overweight and with increased deposition of regional fat in the neck, which may predispose to the development of metabolic alterations. However, no metabolic changes related to increased adiposity were found. In addition, females accumulated more fat than males.

Keywords: exercise, high performance, morphometric measures, overweight.

Resumo

O presente estudo teve como objetivo avaliar o escore de condição corporal (ECC) e perfil metabólico dos participantes finalistas da prova do Freio de ouro, separados por sexo. Esse estudo avaliou 84 animais, 43 fêmeas não-gestantes e 41 garanhões da raça Crioula, os quais foram realizadas as medidas morfométricas de ECC, escore de crista (CNS), avaliação de tamanho de gordura na crista e tamanho de gordura da base da cola, sendo que a partir dessas medidas foi realizada determinação de porcentagem de gordura corporal (%GC), índice de massa corporal(IMC), massa gorda (MG), massa gorda livre(MGL), relação circunferência torácica e altura (CT:AC) e relação circunferência de pescoço e altura (CP:AC). Desses 84 animais, 53 foram coletadas amostra de sangue para avaliação de triglicerídeos, colesterol, lipoproteína de baixa densidade (LDL), lipoproteína de alta densidade (HDL), lactato desidrogenase (LDH), cretina quinase (CK) e adiponectina. Com relação aos escores, 21% dos participantes foram classificados com sobrepeso, e 49% com CNS≥3. Foi encontrada maior deposição de gordura na base da cauda e no pescoço nas fêmeas. As fêmeas também apresentaram maior %GC, MG e relação CT:AC e menor CP:AC quando comparadas aos machos. Das variáveis metabólicas, as fêmeas apresentaram maiores concentrações de LDH, LDL, CK, e menor concentração de adiponectina que os machos. Não foi observado diferença nas medidas morfométricas dos participantes classificados para o último dia de competição dos que foram desclassificados. A partir desse estudo foi possível observar que apesar da prova do Freio de Ouro ser de alta exigência física e de preparo dos participantes, uma porcentagem dos participantes encontravam-se com sobrepeso e com aumento de deposição de gordura regional no pescoço, o que pode predispor ao desenvolvimento de alterações metabólicas. No

entanto, não foi encontrada nenhuma alteração metabólica relacionada ao aumento de adiposidade. Além disso, as fêmeas acumulam mais gordura do que os machos.

Palavras-chave: exercício, alta performance, medidas morfométricas, sobrepeso.

Introduction

The Crioulo breed originated from animals brought from the Iberian Peninsula, specifically, from Andalusian and Spanish native horses, that have become adapted to adverse conditions in the territories of South America developing characteristics such as rusticity and resistance (ABCCC, 2022). The "Freio de Ouro" first functional competition officially originated in 1982. Since then, the competition is considered a primordial step for the selection of animals within the breed. It consists of seven stages, in which each group, horse and rider, need to obtain a score, which, when added together, will indicate the great Champion (ABCCC, 2022). Gender separation is preconized, so males and females do not compete together.

With the rise of the breed and the aim of functional and morphological improvement, the management of these animals was changed. A shift to more intensive breeding and an increase in hypercaloric diets rich in complex carbohydrates has been observed, bringing various consequences such as obesity, dental problems, orthopedic conditions, and colic (Frank et al., 2011; Amaral et al., 2017).

Of these conditions, obesity is nowadays considered a growing problem. It is associated with important metabolic dysfunctions that can lead to consequences that pose a risk to the well-being and are life-threatening. Due to its ancestry and adaptation process, along with the management of these animals, the Crioulo horse can be considered an "easy keeper" breed with a greater tendency to develop overweight and metabolic dysfunctions (Martin-Gimenez et al., 2016).

Furthermore, obesity also affects muscle composition in the body and is known to increase energy requirements limiting sports performance (Kearns et al., 2002). The increase in body fat percentage (%BF) has also been shown to negatively influence the performance of sports horses by causing asymmetry of movement and longer recovery time after exercise (Jansson et al., 2021). Thus, the aims of the present study were: i) to evaluate the body condition of Crioulo horses classified for the finals of the "Freio de Ouro" competition and ii) to evaluate the metabolic variables of the participants according to gender.

Materials and Methods

Animals

This study was carried out during the Freio de Ouro competition, in Esteio, Rio Grande do Sul, Brazil. Eighty-four (84), 43 non-pregnant females with an average age of 7,31±0,23 years old, and 41 stallions with an average age of 8,04±0,27 years old were evaluated. All these animals underwent an admission process on the first day, which includes checking documentation, carrying out standard morphometric measurements of the breed, and mandatory veterinary inspection, to then be enabled to participate in the competition finals. The mandatory veterinary inspection evaluates healthy condition of the horses and lameness status. All the horses admitted must be in perfectly healthy condition, having no open wounds and no lameness of any degree. Of

the Eighty-four animals, fifty-three (53) were also evaluated for metabolic profile (24 non-pregnant females and 28 stallions). All evaluations and sampling were carried out with the owner's and/or veterinarian's consent. All procedures performed were also approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (CEEA-UFPel), protocol number 008501/2022-14.

Morphometric measurements

After completing the admission process, the morphometric assessment was performed. Body condition score (BCS) and crest neck score (CNS) were assessed by two trained observers based on the 9-point scale described by Henneke et al. (1983), and the 5-point scale described by Carter et al., (2009), respectively. Neck circumference was performed at 50% of its length in a relaxed position. Thorax circumference was performed close to the withers, and both neck and thorax were measured using a standard measuring tape. Subcutaneous measurements of fat depth accumulation on the neck crest and tailhead were performed by ultrasound using a 5.0 MHz linear transducer (Sonoscape A5®) as described before (Carter et al., 2009; Gentry et al., 2016). Briefly, crest neck fat thickness was measured with the neck in a normal upright position, at 50% of the neck length from identification of the nuchal ligament. Tailhead fat was measured 7 cm to the right side from the middle plane at the gluteal region. Body weight and height were carried out by the technical team responsible for the event and were available to the research.

From the morphometric measurements, the following formulas to assess body fat and body mass composition were performed: Body mass index (BMI)= (weight(kg)/height(m²)); the ratio between girth circumference and height at the withers (GC: HW); the ratio between neck circumference and height at the withers (NC: HW); % body fat (%BF=5.47x tailhead fat (cm) + 2.47); fat mass (FM=%body fat*Weight(kg)) and free fat mass (FFM= fat mass – weight) (Fonseca et al., 2013; Jensen et al., 2016; Jansson et al., 2021).

Metabolic assay

For metabolic evaluation, owners of fifty-three (53) animals allowed blood collection, which was performed by the veterinarian through puncture of the external jugular vein with a vacutainer® system in red top tubes. The samples were stored until clot formation for about one hour and then were centrifuged at 800g for 10 minutes, the serum was separated into aliquots in 2mL eppendorfs and stored at -20º for further analysis. Triglycerides, cholesterol, LDH, LDL, HDL, and CK analyses were performed using commercial kits in an automatic analyzer (Labmax Plenno®), and adiponectin was performed using a commercial ELISA kit (Horse ADP(Adiponectin) ELISA Kit – FineTest®). All metabolic assays were performed at the biochemistry laboratory of the Research, Teaching, and Extension Nucleus in Livestock (NUPEEC) of the Federal University of Pelotas (UFPel).

Statistical analysis

GraphPad Prism 5® software was used for statistical analysis. Frequency distribution of BCS and CNS was performed. A descriptive analysis of all animals' morphometric measurements and metabolic profile variables and separated by sex was performed. The averages were compared by the unpaired T-test for the

normally distributed variables and the non-normally distributed variables were compared by the Mann-Whitman test. Pearson correlation coefficient was performed between morphometric and metabolic variables.

The measurements of BCS, CNS, tailhead fat, neck crest height, and %BF of the animals that were classified on the last day of the competition were also compared to those that were not classified. A significance level of 5% was considered and the data are presented as mean±S.E.M.

Results and Discussion

To the best of the authors' knowledge, this is the first study evaluating body fat composition and metabolic status of Crioulo horses' finalists of the Freio de Ouro competition. Freio de Ouro competition is considered to be of high demand and physical preparation (Garcia et al., 2020), even so, twenty-one percent (21%) of the competing animals were considered overweight (7/9), and an even higher percentage (49%) were identified with a CNS ≥3/5, which indicates a greater probability of developing metabolic dysfunction (Fitzgerald et al., 2019). Muñoz et al. (2019) also evaluated the body condition of Chilean horses in a rodeo event and found that 50% of participants were 7/9, and a small percentage (6.3%) were obese (BCS=8/9). The morphometric data of the 84 animals evaluated and separated by sex are described in Table 1

The body condition score assessment described by Henneke et al. (1983) is the most widely used system but is considered a subjective and less sensitive assessment to identify the deposition of general or local body fat in the horse and differences between breeds must be considered. The average BCS in this study was similar to what was described for other breeds (6.10± 0.06, min 5 - max 7) (Martin-Gimenez et al., 2018; Di Filippo et al., 2019)

The 5-point CNS system is also a subjective assessment of fat deposits along the neck but has been used as a better predictor of metabolic dysfunction in equines (Carter et al., 2009; Fitzgerald et al., 2016). Ponies with CNS≥3/5 were five times more susceptible to developing insulin dysregulation and presented higher triglycerides concentration (Fitzgerald et al., 2016). A high percentage of horses (49%) in the present study had the same score, however, metabolic variables evaluated (cholesterol, triglycerides, and LDL) were within the reference range (Table 2) (Kaneko et al., 1997; Frank et al., 2006; Elzinga et al., 2016), except HDL, which was increased in both males and females, what could be secondary to a high-fat diet usually provided to athlete horses (Geelen et al., 2001). Also, no correlation of metabolic variables with morphometric measures was found in males and females in the present study.

Other measures have also been considered more objective for obesity identification in the horse, such as the percentage of body fat, which has a high relationship with the amount of fat tissue in horses (Westervelt et al., 1976). Average body fat was higher in Crioulo horses than in thoroughbreds (Fonseca et al., 2016) and Colombian Passo fino horses (Cabrera & Valencia, 2020) and similar to the values described for Icelandic horses (Jansson et al., 2021). Body fat (%) was also found to be negatively correlated to FFM in this study (Tables 3 and 4), which is indicative of muscle content in the body. A greater FFM suggests a larger muscle mass and consequently greater strength potential while an increased percentage of body fat is described to negatively affect the performance because increases energy requirements (Kearns et al., 2002).

Carter et al. (2009) identified the relationships between girth circumference: height at the withers and neck circumference: height at the withers as better and more objective measures to assess general body fat and regional fat deposits, respectively. Both these ratios are reported to increase following the increase in BCS, and both have a cutoff value of 1,26 and 0,63, respectively for horses. Average GC: HW in this study was 1,26 and NC: HW was 0,67. These values were based on another breed, so careful evaluation must be taken into account, considering that the mean BCS in the animals evaluated was compatible with moderate/normal BCS horses. Jensen et al. (2016) suggested different cutoff values depending on the breed.

Regarding gender differences in morphometric evaluation, females were found to have greater adipose tissue deposits in the gluteal region, and in the neck crest and also had greater measures of %BF, FM, and GC: HW, and lower NC: HW than males. Considering all these measures, it can be inferred that the Crioulo mares' finalists of the competition tend to accumulate more adipose tissue in general than males which could also affect performance (Kearns et al., 2002; Fonseca et al., 2012; Klein et al., 2020). Interestingly, despite the crest neck thickness being greater in mares, NC: HW was increased in the stallions. This ratio is used to assess regional fat deposits and, in this case, could be related to the phenotypic characteristics of the gender itself as reported by another author (Martin-Gimenez et al., 2018). In one study, higher subcutaneous adipose deposits and neck morphometrics were found in Andalusian stallions and were related to sex dimorphism (Martin-Gimenez et al., 2018). On the other hand, Jensen et al. (2016) and Cabrera & Valencia (2020), found no gender influence on morphometric evaluations. In another study, despite NC: HW being higher in a group with an obese body condition score, no correlation with the fat deposit in the neck was found (Fitzgerald et al., 2016), differing from the present study in which this measure was associated with fat depth on the neck in males and CNS in females.

Of the total evaluated animals, 29.76% (25/84) were classified to compete on the last day of competition. Of this total, 80% (20/25) had a BCS between 5 and 6/9 and 20% (5/25) had a BCS of 7/9. No difference was observed between the animals that passed to the last day to those that did not in BCS (6.04± 0.12; 6.13± 0.08), CNS (2.64 ±0.09; 2.57±0.06), neck crest thickness (7.97±0.27 cm; 8.91±0.27 cm) and tailhead fat (2.19±0.14 cm; 2.07±0.07 cm), %BF (14,74±0,70; 13,75±0,42), respectively. Recently, a study identified that an increase of 5 to 8 % in weight, BCS, and body fat reduces lactate removal, glucose availability, and recovery from exercise, decreasing performance and inducing locomotion asymmetry (Jansson et al., 2021). In addition, an increase in body weight can negatively affect the locomotor apparatus, which can be amplified after the exercise is finished (Jansson et al., 2021).

Creatine Kinase concentration in both males and females was smaller in this study than what was reported before in healthy Crioulo horses in the pasture and exercise conditions (Da Cas et al., 2000; Lacerda et al., 2006, Amaral et al., 2013) and in other breeds (Stuchi et al., 2019). CK is an enzyme related to muscle function. Non-pathological states as exercises can influence its release, suggesting certain cellular damage (Lacerda et al., 2006). Exercise conditioning programs that athlete horses are submitted to can help in CK concentrations preventing high cellular damage and the release of this enzyme profusely (Siciliano et al., 1995; MacLeay, 2010). Females presented higher CK concentration than males, which is in agreement with what is described by another author (Da Cas et al., 2000). It is suggested that females could have increased enzymatic

activity or slower removal rates from the circulation (Da Cas et al., 2000), however, this association is still controversial (MacLeay,2010).

LDH was also increased in both sexes (Kaneko et al., 1997) and was higher in females than males. LDH is also a muscle enzyme, related to CK, that increases secondary to exercise and muscle damage. Circulating CK half-life is smaller than LDH, so LDH concentration remains increased for at least 48 hours after cell damage or muscle leakage (Teixeira-Neto et al., 2008). In one study evaluating Crioulo horses in a 750 km gait competition, increases of CK and LDH of 2614% and 717% at the end of the competition were observed, respectively, demonstrating the high muscular demand of the breed in this kind of competition (Amaral et al., 2013). The Freio de Ouro competition is also a high-demand competition but more functional and evaluates movement perfection demanding a greater time of training and conditioning, which differs from the 750km gait competition in which the animals have less time to prepare. Furthermore, another study by our research group on Crioulo horses competing in Freio de Ouro, identified higher values of CK and LDH before starting the competition than at the end, which indicates that those animals had higher physical demand within seven days prior (Amaral et al., 2014).

Females had smaller adiponectin concentrations than males which could be related to the greater adiposity observed in this group represented by morphometric measures. In other species, the opposite is observed, females have a greater concentration of adiponectin, which is suggested to occur because testosterone can inhibit its secretion (Xu et al., 2005; Song et al., 2014). No association of adiponectin concentration with morphometric measures was observed. Kearns et al. (2006) identified a negative correlation between adiponectin with percent body fat and fat mass in horses. Adiponectin has anti-inflammatory properties and enhances insulin sensitivity (Wooldridge et al., 2012). Adiponectin is also discussed as not being altered only in over-conditioned horses but is decreased in horses with abnormal insulin regulation. Furthermore, the animals evaluated had a continuous training program to be able to participate in the competition, and exercise is known to improve adiponectin production in humans (Becic et al., 2018) and in horses (Bamford et al., 2019).

Conclusion

The participants of the Freio de Ouro competition had moderate BCS with 21% of them being overweight. No alterations were found in the metabolic markers related to adiposity. No difference in morphometric measures was observed in the participants classified for the last day of the competition from those not. The participants had increased muscle enzymes which are related to the competition itself. Females were noticed to accumulate more fat than males.

Conflict of Interest

The authors have no competing interests.

Acknowledgments

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Table 1.

Morphometric measurements of participants in the Freio de Ouro competition.

	General	*Females*	*Males*
Weight (kg)	490.1±4.26	501.2±7,09[a]	478.6±3.83[b]
BCS	6.10± 0.06	6.2± 0.10	6.0 ± 0.08
CNS	2.59± 0.05	2.61± 0.08	2.57± 0.07
Tailhead fat (cm)	2.11± 0.99	2.42± 0.95[a]	1.80 ± 0.67[b]
Crest neck thickness (cm)	8.64± 0.89	8.16± 0.27[a]	7.38 ±0.16[b]
GC: HW	1.26 ±0.00	1.28±0.00[a]	1.25±0.00[b]
NC: HW	0,67±0.46	0,63±0.35[a]	0,70±0.42[b]
Body mass index (BMI)	304.2±2.03	303.6±3.74	304.7±1.62
% BF	14.01±0.36	15,56±0.51[a]	12.59±0.41[b]
FM	69.04±2.07	79,10±3.02[a]	58,99±1,81[b]
FFM	421.0±3.60	422.4±6.18	419.6±3.77

*Different letters indicate statistical differences.

Table 2.

Concentrations of triglycerides, cholesterol, LDH, LDL, CK, and Adiponectin in participants in the Freio de Ouro competition, separated by gender.

	Females	*Males*
Triglicerídeos (mg/dL)	22.25 ± 1.994	25.00 ± 2.846
Cholesterol total (mg/dL)	99.21 ± 2.589	93.10 ± 3.227
LDH (U/L)	637.7 ± 31.40[a]	502.9 ± 20.54[b]
LDL mg/dL	37.08 ± 2.475[a]	29.69 ± 2.129[b]
HDL (mg/dL)	234.0 ± 9.393	219.7 ± 9.939
CK (U/L)	66.83 ± 14.46[a]	29.83 ± 4.329[b]
Adiponectina (ng/mL)	5.980 ± 0.2029[a]	6.698 ± 0.2421[b]

*Different letters indicate statistical differences.

Table 3.

Significant (p<0,05) correlation coefficients (r) between morphometric measures and calculated measures in females.

Females	r
Weight x GC: HW	0.59
FFM x Tailhead fat	-0.51
CNS x NC: HW	0.54
CNS x Neck crest thickness	0.54
CNS x BCS	0.53
GC: HW x FFM	0.46
%BF x FFM	-0.52

Table 4.

Significant (p<0,05) correlation coefficients(r) between morphometric measures and calculated measures in males.

Males	r
Neck crest thickness x NC: HW	0.40
Tailhead fat x %BF	0.46
CNS x Neck crest thickness	0.58
%BF x FFM	-0.54
%BF x FM	0.50
%BF x Neck crest	0.40

4 Considerações Finais

A partir do presente trabalho foi possível observar que éguas Crioulas gestantes com escore corporal BCS 5-6 e BCS≥7 vacinadas contra o EHV-1, responderam a vacinação produzindo anticorpos específicos, com duração de pelo menos 155 dias, porém não apresentaram efeito *boosting* na segunda dose. Não foi observado influência da gestação ou da obesidade na resposta imune humoral.

. Na avaliação dos participantes do Freio de Ouro, foi identificado uma porcentagem de animais apresentando sobrepeso, sendo que as fêmeas apresentaram maiores medidas corporais que os machos. Não foi identificado alterações metabólicas relacionadas ao aumento de escore.

A obesidade atualmente tem sido retratada como um problema crescente dentro das criações de equinos de diversas raças. O perfil do animal mais arredondado e preenchido tem sido valorizado em provas morfológicas e pelos próprios criadores em diversas raças. Na raça Crioula não é diferente, um maior número de animais com sobrepeso e obesidade é observado nos diversos sistemas de criação.

Apesar disso, a partir das observações do presente estudo e dos demais trabalhos realizados pelo grupo ClinEq sobre a raça, é possível inferir que o cavalo Crioulo se mantém estável metabolicamente em condições de sobrepeso, o que difere das características mais comuns vistas nas demais raças. O estado metabólico de animais Crioulos com sobrepeso se altera diante de alguma injúria ou stress importante. Isso pode estar ligado a adaptação e rusticidade a qual a raça é reconhecida, porém ainda não se sabe com certeza a causa dessa grande diferença das demais raças. Devido a essas observações e ao crescimento e expansão da raça Crioula, tornou-se relevante manter esses animais em um controle perante avaliações morfométricas e metabólicas, tendo em vista a predisposição da raça ao acúmulo de gordura.

Os trabalhos expostos nessa tese são uma pequena parte de vários estudos realizados sobre o assunto, o qual desde o período de residência se tornou a minha principal linha de pesquisa junto ao grupo ClinEq. Além desse assunto, foi possível a

participação e desenvolvimento de diversos outros estudos com foco em assuntos de importância na medicina de equinos abrangendo as principais linhas de pesquisa do grupo.

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Anexos

1ª página:

Anexo I - Documento da Comissão de Ética e Experimentação Animal

Artigo 1

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **1/2020/CEEA/REITORIA**
PROCESSO Nº 23110.034960/2019-40

Certificado

Certificamos que a proposta intitulada **"Avaliação imunológica de éguas com diferentes escores corporais gestantes e não gestantes e de seus respectivos potros"**, registrada com o nº 23110.034960/2019-40, sob a responsabilidade de Carlos Eduardo Wayne Nogueira - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), 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 de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **17 de dezembro de 2019**.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	07/01/2020 a 30/09/2020
Espécie/linhagem/raça	Equina/Crioula
Nº de animais	70
Idade	45 entre 2-15 anos e 25 entre 0-6 meses
Sexo	Machos e Fêmeas
Origem	Criatório de raça Crioula em Santa Vitória do Palmar/RS

Código para cadastro nº **CEEA 34960-2019**

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M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Certificado

Certificamos que a proposta intitulada **"Perfil metabólico, condição corporal e avaliação radiográfica de cavalos Crioulos finalistas do ciclo funcional e morfológico da ABCCC"**, registrada com o nº **23110.008501/2022-14**, sob a responsabilidade de **Carlos Eduardo Wayne Nogueira** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), 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 de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética no Uso de Animais da Universidade Federal de Pelotas.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	Início = 14/09/2022 Término = 20/06/2024
Espécie/linhagem/raça	Equina / Crioula
Nº de animais	**134**
Idade	2-10 anos
Sexo	Machos e Fêmeas
Origem	Associação Brasileira de Criadores de Cavalos Crioulos-Parque de Exposições Assis Brasil, Esteio/RS.

Código para cadastro nº **CEUA 008501/2022-14**

Priscila Marques Moura de Leon

Coordenadora da CEUA