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

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

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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 found to accumulate more fat than males.

Abstract

The aim of this study was to assess the body condition score (BCS) and metabolic profile of finalists in the Freio de Ouro competition, stratified by sex. This study examined 84 Crioulo horses, comprising 43 non-pregnant mares and 41 stallions. Morphometric measurements, including BCS, cresty neck score (CNS), neck and heart girth, and assessments of fat depth at the neck and at the tail base, were conducted. Additional derived measurements such as the percentage of body fat (%BF), body mass index (BMI), fat mass (FM), fat-free mass (FFM), heart girth:height ratio (HG:HW), and neck circumference:height ratio (NC:HW) were calculated. A subgroup of 53 animals was selected for the evaluation of metabolic parameters, including triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), creatine kinase (CK), and adiponectin.In terms of BCS, 21% of participants were classified as overweight, with 49% having CNS≥3. Females exhibited greater fat deposition at the tail base and neck, along with higher %BF, FM, and HG:HW ratio, and lower NC:HWcompared to males. Among metabolic variables, females showed higher concentrations of LDH, LDL, CK, and lower concentrations of adiponectin than males. No differences were observed in morphometric measurements between

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participants that qualified for the final day of the competition and those which were disqualified. The study revealed that despite the rigorous physical demands and preparation required for the Freio de Ouro competition, a percentage of participants were overweight, displaying increased regional fat deposition in the neck. Although no metabolic changes related to increased adiposity were identified, the findings indicate a predisposition to the development of metabolic alterations. Additionally, females exhibited a greater accumulation of fat than males.
Key words: Exercise. High performance. Morphometric measurements. 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: Marsupial. *Theileria.* Carrapatos. Pulgas. Vetores.

Introduction

The Crioulo breed has its origins in animals brought from the Iberian Peninsula, specifically Andalusian and Spanish native horses. Over time, these horses have adapted to the challenging conditions of South American territories, developing characteristics such as hardiness and resistance (Associação Brasileira de Criadores de Cavalos Crioulos [ABCCC], 2022). The Freio de Ouro' first functional competition, was officially established in 1982 and has since then been considered a crucial step in the breed's animal selection process. Comprising nine stages, the competition

requires each group, consisting of a horse and rider, to obtain scores. The cumulative scores determine the Grand Champion (ABCCC, 2022), with sex segregation ensuring that males and females do not compete together.

With the rising prominence of the Crioulo breed and the goal of functional and morphological improvement, a shift in the management of these animals has been observed. There has been a move towards more intensive breeding and adoption of hypercaloric diets rich in complex carbohydrates, leading to various consequences such as obesity, dental problems, orthopedic conditions, and colic (Frank, 2011; Amaral et al., 2017).

Among these conditions, obesity is currently recognized as a growing problem. It is linked to essential metabolic dysfunctions that pose risks to well-being and can be life-threatening. Due to its ancestral lineage, adaptation process, and management practices, the Crioulo horse is considered an "easy keeper" breed with a higher tendency to develop overweight and metabolic dysfunctions (Martin-Gimenez et al., 2016).

Moreover, obesity affects muscle composition and is known to increase energy requirements, limiting sports performance (Kearns et al., 2002). An elevated body fat percentage (%BF) has been shown to negatively impact the performance of sports horses by causing movement asymmetry and longer recovery times after exercise (Jansson et al., 2021). Therefore, the objectives 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 based on sex.

Materials and Methods

Animals

This study was conducted during the Freio de Ouro competition in Esteio, Rio Grande do Sul, Brazil. A total of 84 horses were evaluated, consisting of 43 non-pregnant mares with an average age of 7.31±0.23 years and 41 stallions with an average age of 8.04±0.27 years. All animals underwent an admission process on the first day, involving documentation verification, standard morphometric measurements of the breed, and mandatory veterinary inspection. This process enabled them to participate in the competition finals. The mandatory veterinary inspection assessed the overall health and lameness status of the horses. Admitted horses were required to be in perfect health, with no open wounds and no degree of lameness. Out of the 84 animals, 53 were also subjected to a metabolic profile evaluation (24 non-pregnant mares and 28 stallions). All evaluations and samplings were conducted with the owner's and/or veterinarian's consent, and all procedures were approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (CEEA-UFPel, approval no. 008501/2022-14).

Morphometric measurements

Following the admission process, a morphometric assessment was conducted. Body condition score (BCS) and cresty neck score (CNS) were evaluated by two trained observers using the nine-point scale described by Henneke et al. (1983) and the five-point scale described by Carter et al.

(2009), respectively. Neck circumference was measured at 50% of its length in a relaxed position, while heart girth was measured close to the withers, using a standard measuring tape. Subcutaneous measurements of fat depth on the neck crest and tailhead were performed using ultrasound with a 5.0 MHz linear transducer (Sonoscape A5®), as described previously (Carter et al., 2009; Gentry et al., 2004). Shortly after, fat thickness at the neck crest was measured with the neck in a normal upright position, at 50% of the neck length from the identification of the nuchal ligament. Tailhead fat was measured 7 cm to the right side from the middle plane in the gluteal region. Body weight was measured based on heart girth, and height was measured with a horse measuring stick. Both measurements were conducted by the technical team responsible for the event and were made available for research purposes.

Morphometric measurements were employed to derive formulas for assessing body fat and body mass composition. The following calculations were thus performed: Body Mass Index (BMI) = (Weight(kg)/ Height(m^2)); Ratio between heart girth and height at the withers (HG:HW); Ratio between neck circumference and height at the withers (NC:HW); %Body fat (%BF = 5.47 ×Tailhead fat (cm) + 2.47); Fat mass (FM = %BF× Weight(kg)); and Fat-free mass (FFM = FM - Weight (kg)) (Fonseca et al., 2013; Jensen et al., 2016; Jansson et al., 2021).

Metabolic assay

For metabolic evaluation, blood collection from 53 animals was permitted by their owners, conducted before the competition by a veterinarian through puncture of the external jugular vein with a Vacutainer® system in red top tubes. The samples were stored until clot formation for approximately one hour and then centrifuged at 800g for 10 min; the serum was separated into aliquots in 2 mL Eppendorfs and stored at -20ºC for subsequent analysis. Triglycerides, cholesterol, LDH, LDL, HDL, and CK analyses were performed using commercial kits in an automatic analyzer (Labmax Plenno®), and adiponectin was assessed using a commercial ELISA kit (Horse ADP [Adiponectin] ELISA Kit - FineTest®). All metabolic assays were conducted at the biochemistry laboratory of the Research, Teaching, and Extension Nucleus in Livestock (NUPEEC) of the Federal University of Pelotas (UFPel).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5® software. The frequency distribution of BCS and CNS was determined. Descriptive analyses of all animals' morphometric measurements and metabolic profile variables, separated by sex, were performed. Means were compared using the unpaired T-test for normally distributed variables, and the Mann-Whitney test was applied for non-normally distributed variables. Pearson correlation coefficients were calculated between morphometric and metabolic variables.

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

Results and Discussion

To the best of the authors' knowledge, this study represents the first examination of body fat composition and metabolic status in Crioulo horses finalists in the Freio de Ouro competition. Despite being a competition recognized for its high physical demand and preparation (Garcia et al., 2020), 21% of the participating animals (7/9) were considered overweight, with an even higher percentage (49%) exhibiting a CNS ≥3/5, indicating an increased likelihood of developing metabolic dysfunction (Fitzgerald et al., 2019). A study by Muñoz et al. (2019) assessing Chilean horses in a rodeo event found that 50% of participants had a BCS of 7/9, with a small percentage (6.3%) being obese (BCS=8/9). Table 1 describes the morphometric data of the 84 animals evaluated and separated by sex.

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]
Neckcrestthickness (cm)	8.64± 0.89	8.16± 0.27[a]	7.38 ±0.16[b]
HG: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.BCS (body condition score); CNS (cresty neck score); HG: HW (ratio between heart girth and height at the withers); NC:HW (ratio between neck circumference and height at the withers); %BF (% body fat); FM (fat mass); FFM (fat-free mass).

The body condition score (BCS) assessment proposed by Henneke et al. (1983) is the most commonly employed system, yet it is considered subjective and less sensitive in identifying the deposition of general or local body fat in horses, with variations between breeds. The average BCS in this study was similar to that described for other breeds (6.10± 0.06; min 5 - max 7) (Martin-Gimenez et al., 2018; Di Filippo et al., 2019).

The five-point CNS system, another subjective assessment, has been utilized as a better predictor of metabolic dysfunction in equines (Carter et al., 2009; Fitzgerald et al.,

2019). Ponies with CNS≥3/5 were found to be five times more susceptible to developing insulin dysregulation and exhibited higher triglyceride concentrations (Fitzgerald et al., 2019). In this study, a high percentage of horses (49%) had the same score. However, the evaluated metabolic variables (cholesterol, triglycerides, and LDL) were within the reference range (Table 2) (Kaneko et al., 1997; Frank et al., 2006; Elzinga et al., 2016), except for HDL, which was elevated in both males and females, possibly due to the high-fat diet typically provided to athlete horses (Geelen et al., 2001). Moreover, no correlation of metabolic variables with morphometric measurements was found in males and females in the current study.

Table 2
Concentrations of triglycerides, cholesterol, LDH, LDL, CK, and adiponectin in participants in the Freio de Ouro competition, separated by sex

	Females	*Males*
Triglycerides (mg/dL)	22.25 ± 1.994	25.00 ± 2.846
Total cholesterol (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]
Adiponectin (ng/mL)	5.980 ± 0.2029[a]	6.698 ± 0.2421[b]

*Different letters indicate statistical differences. LDH (lactate dehydrogenase); LDL (low-density lipoproteins); HDL (high-density lipoproteins); CK (creatine kinase).

Other more objective metrics for identifying obesity in horses include the percentage of body fat, which has a strong correlation with the amount of fat tissue (Westervelt et al., 1976). The average body fat in Crioulo horses was higher than in Thoroughbreds (Fonseca et al., 2013) and Colombian Paso Fino horses (Cabrera & Valencia, 2020), and similar to values described for Icelandic horses (Jansson et al., 2021). Body fat (%) was negatively correlated with FFM in this study (Tables 3 and 4), indicative of muscle content. Greater FFM suggests a larger muscle mass and, consequently, greater strength potential, while an increased percentage of body fat is known to negatively affect performance due to elevated energy requirements (Kearns et al., 2002).

Table 3
Significant (p<0.05) correlation coefficients (r) between morphometric measurements and calculated measurements in females

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

HG:HW (ratio between heart girth and height at the withers); FFM (fat-free mass); CNS (cresty neck score); NC: HW (ratio between neck circumference and height at the withers); BCS (body condition score); %BF (% body fat).

Table 4
Significant (p<0.05) correlation coefficients(r) between morphometric measurements and calculated measurements in males

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

NC:HW (ratio between neck circumference and height at the withers); %BF (% body fat); CNS (cresty neck score); FFM (fat-free mass), FM (fat mass).

Carter et al. (2009) proposed the heart girth:height at the withers and neck circumference:height at the withers ratios as better and more objective metrics for assessing general body fat and regional fat deposits, respectively. Both ratios are reported to increase with higher BCS, with cutoff values of 1.26 and 0.63, respectively, for horses. The average HG:HW in this study was 1.26, and NC:HW was 0.67. These values, derived from another breed, warrant careful consideration, especially since the mean BCS in the evaluated animals was compatible with moderate/normal BCS horses. Jensen et al. (2016) suggested different cutoff values depending on the breed.

Concerning sex differences in morphometric evaluation, females exhibited greater adipose tissue deposits in the gluteal and neck crestregions. They also showed

higher values of %BF, FM, and HG:HW, along with a lower NC:HW compared to males. Taking all these metrics into account, it can be deduced that Crioulo mare finalists in the competition tend to accumulate more adipose tissue in general than males, potentially impacting performance. This variation in adipose tissue distribution may be linked to differing concentrations of sexual steroids, leading to females having a lower percentage of muscle mass and a higher percentage of fat compared to males (Kearns et al., 2002; Fonseca et al., 2013; Klein et al., 2020). Interestingly, despite mares having greater neck crest thickness, NC:HW was higher in stallions. This ratio is utilized to assess regional fat deposits and, in this instance, may be associated with the inherent phenotypic characteristics of sex, as reported by another author (Martin-Gimenez et al., 2018). In a study, Andalusian stallions exhibited higher subcutaneous adipose deposits and neck morphometrics related to sex dimorphism (Martin-Gimenez et al., 2018). Conversely, Jensen et al. (2016) and Cabrera and Valencia (2020) found no sex influence on morphometric evaluations. In another study, despite higher NC:HW in a group with an obese body condition score, no correlation with neck fat deposit was found (Fitzgerald et al., 2019), differing from the present study in which this measurement was associated with fat depth on the neck in males and CNS in females.

Of the total animals evaluated, 29.76% (25/84) were classified to compete on the last day of the 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 differences were observed between the animals that progressed to the last day and those that did not in terms of 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), tailhead fat (2.19±0.14 cm; 2.07±0.07 cm), %BF (14.74±0.70; 13.75±0.42), and weight (445.10±9.17 kg; 435.10±4.07 kg), 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). Moreover, an increase in body weight can negatively impact the locomotor apparatus, which may be amplified after exercise is completed (Jansson et al., 2021).

Creatine kinase concentration in both males and females was smaller in this study than reported previously in healthy Crioulo horses in pasture and exercise conditions (Da Cás et al., 2000; Lacerda et al., 2006; Amaral et al., 2013) and in other breeds (Stuchi et al., 2019). Creatine kinase is an enzyme related to muscle function. Non-pathological states like exercise can influence its release, indicating certain cellular damage (Lacerda et al., 2006). Exercise conditioning programs that athlete horses undergo can help maintain CK concentrations, preventing excessive cellular damage and the profuse release of this enzyme (Siciliano et al., 1995; MacLeay, 2010). Females exhibited higher CK concentrations than males, consistent with the findings of another author (Da Cás et al., 2000). It is suggested that females could have increased enzymatic activity or slower removal rates from the circulation (Da Cás et al., 2000); however, this association remains controversial (MacLeay, 2010).

Lactate dehydrogenase levels were elevated in both sexes, consistent with findings in the literature (Kaneko et al., 1997), and were higher in females compared to males. Lactate dehydrogenase, a muscle enzyme related to CK, increases secondary to exercise and muscle damage. The circulating half-life of CK is smaller than LDH, so the LDH concentration remains elevated for at least 48 h after cell damage or muscle leakage (Teixeira et al., 2008). In a study evaluating Crioulo horses in a 750 km gait competition, there were increases of 2614% and 717% in CK and LDH, respectively, at the end of the competition, highlighting the high muscular demand of the breed in this type of event (Amaral et al., 2013). The Freio de Ouro competition is also demanding, but it focuses more on functionality and evaluates movement perfection, requiring a longer time of training and conditioning. This differs from the 750-km gait competition, where animals have less time to prepare. Another study by our research group on Crioulo horses in Freio de Ouro identified higher values of CK and LDH before starting the competition than at the end, indicating higher physical demand within seven days prior (Amaral et al., 2013).

Mares exhibited lower adiponectin concentrations than males, potentially linked to the greater adiposity observed in this group based on morphometric measurements. In contrast to other species, where females tend to have higher adiponectin concentrations, the observed pattern in horses may be influenced by testosterone inhibiting its secretion (Xu et al., 2005; Song et al., 2014). No association between adiponectin concentration and morphometric measurements was observed. Kearns et al. (2006) identified a negative correlation between adiponectin and percent body fat and fat mass in horses. Adiponectin, known for its anti-inflammatory properties and role in enhancing insulin sensitivity (Wooldridge et al., 2012), is discussed as being altered not only in over-conditioned horses but also in those with abnormal insulin regulation. Moreover, the animals in this study had a continuous training programto be able to participate in the competition, and exercise is known to improve adiponectin production in both humans (Becic et al., 2018) and in horses (Bamford et al., 2019).

One limitation of this study was the one-time evaluation of the horses, as it was conducted during the final of the Freio de Ouro competition, the primary selection competition for the breed. More specific metabolic evaluations were not possible to avoid compromising the animals' performance. However, we believe that a single assessment was sufficient for our study. More studies in the Crioulo breed are necessary to elucidate why metabolic markers are not altered in horses with phenotypic characteristics of obesity.

Conclusion

The participants in the Freio de Ouro competition exhibited moderate BCS, with 21% classified as overweight. No alterations were observed in metabolic markers related to adiposity, and no differences in morphometric measurements were noted between participants classified for the last day of the competition and those that were not. Increased muscle enzymes related to the competition itself were observed. Females were found to accumulate more fat than males.

Conflict of interest

The authors have no competing interests.

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References

Amaral, L. A., Marchiori, M., Martins, C. F., Correa, M. N., & Nogueira, C. E. W. (2013). Metabolic evaluation of Crioulo horses participating in competitions of 750 km. *Pesquisa Veterinária Brasileira, 33*(12), 1471-1477. doi: 10.1590/S0100-736X2013001200013

Amaral, L. A., Marchiori, M., Moraes, B. S., Finger, I., Santos, R. S., & Nogueira, C. E. W. (2017). Relação entre adiposidade, perfil energético, proteínas inflamatórias e lesões osteoarticulares em equinos jovens sobre diferentes sistemas de criação. *Pesquisa Veterinária Brasileira, 37*(2), 115-120. doi: 10.1590/S 0100-736X2017000200004

Associação Brasileira de Criadores de Cavalos Crioulos (2022). *História da ABCCC.* https://www cavalo crioulo.org. br/institucional/historia.

Bamford, N. J., Potter, S. J., Baskerville, C. L., Harris, P. A., & Bailey, S. R. (2019). Influence of dietary restriction and low-intensity exercise on weight loss and insulin sensitivity in obese equids. *Journal of Veterinary Internal Medicine, 33*(1), 280-286. doi: 10.1111/jvim.15374

Becic, T., Studenik, C., & Hoffmann, G. (2018). Exercise increases adiponectin and reduces leptin levels in prediabetic and diabetic individuals: systematic review and meta-analysis of randomized controlled trials. *Medical Sciences, 6*(4), 97 doi: 10.3390/medsci6040097

Cabrera, A. M. Z., & Valencia, N. M. P. C. (2020). Body fat evaluation in Colombian Paso horses: body condition score and morphometric and ultrasound measurements. *Brazilian Journal of Veterinary Research and Animal Science, 57*(4), e171082. doi: 10.11606/issn.1678-4456.bjvras.2020.171082

Carter, R. A., Geor, R. J., Staniar, W. B., Cubitt, T. A., & Harris, P. A. (2009). Apparent adiposity assessed by standardized scoring systems and morphometric measurements in horses and ponies. *Veterinary Journal, 179*(2), 204-210. doi: 10.1016/j.tvjl.2008.02.029

Da Cás, E. L., Rosauro, A. C., Silva, C. A. M., & Brass, K. E. (2000). Concentração sérica das enzimas Creatina Quinase, Aspartato Aminotransferase e Dehidrogenase lática em equinos da raça Crioula. *Ciência Rural, 30*(1), 1-5. doi: 10.1590/S0103-84782000000400011

Di Filippo, P. A., Meireles, M. A. D., Ribeiro, L. M. F., Lannes, S. T., Meireles, N. F. T., Viana, I. S., & Hokamura, H. K. (2019). Influence of exercise, age, body weight, and growth on the development of tarsal osteoarthritis in young Mangalarga Marchador horses. *Journal of Equine Veterinary Science, 80,* 36-39. doi: 10.1016/j.jevs.2019.06.015

Elzinga, S., Wood, P., & Adams, A. A. (2016). Plasma lipidomic and inflammatory cytokine profiles of horses with equine metabolic syndrome. *Journal of Equine Veterinary Science, 40,* 49-55. doi: 10.1016/j.jevs. 2016.01.013

Fitzgerald, D. M., Anderson, S. T., Sillence, M. N., & Laat, M. A. de. (2019). The cresty neck score is an independent predictor of insulin dysregulation in ponies. *PLoS One, 14*(7), e0220203. doi: 10.1371/journal. pone.0220203

Fonseca, R. G., Kenny, D. A., Hill, E. W., & Katz, L. M. (2013). The relationship between body composition, training and race performance in a group of Thoroughbred flat racehorses. *Equine Veterinary Journal, 45*(5), 552-557. doi: 10.1111/evj.12024

Frank, N. (2011). Equine metabolic syndrome. *Veterinary Clinics of North America: Equine Practice, 27*(1), 73-92. doi: 10.1016/j.cveq.2010.12.004. PMID: 2139 2655

Frank, N., Elliott, S. B., Brandt, L. E., & Keisler, D. H. (2006). Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *JAVMA, 228*(9), 1383-1390. doi: 10.2460/javma.228.9.1383

Garcia, C. A. S. C., Martins, C. F., Silva, L. L., Brandi, R. A., Feijó, J. O., Barbosa, A. A., Bruhn, F. R. P., & Corrêa, M. N. (2020). Frequência cardíaca, lactacidemia e gasto energético de equinos da raça Crioula em provas credenciadoras ao Freio de Ouro. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 72*(5), 1631-1638. doi: 10.1590/1678-4162-11741

Geelen, S. N., Jansen, W. L., van Oldruitenborgh-Oosterbaan, M. M., Breukink, H. J., & Beynen, A. C. (2001). Fat feeding increases equine heparin-released lipoprotein lipase activity. *Journal of Veterinary Internal Medicine, 15*(5), 478-481. doi: 10.1892/0891-6640(2001)015<0478:ffiehl>2.3.co;2

Gentry, L. R., Thompson, D. L., Jr., Gentry, G. T., Jr., Del Vecchio, R. P., Davis, K. A., & Del Vecchio, P. M. (2004). The relationship between body condition score and ultrasonic fat measurements in mares of high versus low body condition. *Journal of Equine Veterinary Science, 24*(5), 198-203. doi: 10.1016/j.jevs.2004.04.009

Henneke, D. R., Poiter, G. D., Kreider, J. L., & Yeates, B. F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Veterinary Journal, 15*(4), 371-372. doi: 10./1111/j.2042-3306.1983.tb01826.x

Jansson, A., Gunnarsson,V. Þ, Ringmark, S., Ragnarsson, S., Söderroos, D., Ásgeirsson, E., Jóhannsdóttir, T. R., Liedberg, C., & Stefánsdóttir, G. J. (2021). Increased body fat content in horses alters metabolic and physiological exercise response, decreases performance, and increases locomotion asymmetry. *Physiological Reports, 9*(11), e14824. doi: 10.14814/phy2.14824

Jensen, R. B., Danielsen, S. H., & Tauson, A. H. (2016). Body condition score, morphometric measurements and estimation of body weight in mature Icelandic horses in Denmark. *Acta Veteterinaria Scandinavica, 58*(1), 59. doi: 10.1186/s13028-016-0240-5

Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (1997). *Clinical biochemistry of domestic animals* (5nd ed.). Academic Press.

Kearns, C. F., Mckeever, K. H., Kumagai, K., & Abe, T. (2002). Fat-free mass is related to one-mile race performance in elite Standardbred horses. *The Veterinary Journal, 163*(3), 260-266. doi: 10.1053/tvjl.2001.0656

Kearns, C. F., McKeever, K. H., Roegner, V., Brady, S. M., & Malinowski, K. (2006). Adiponectin and leptin are related to fat mass in horses. *The Veterinary Journal, 172*(3), 460-465. doi: 10.1016/j.tvjl.2005.05.002. PMID: 15996495

Klein, D. J., Anthony, T. G., & McKeever, K. H. (2020). Changes in maximal aerobic capacity, body composition, and running capacity with prolonged training and detraining in Standardbred horses. *Comparative Exercise Physiology, 16*(3), 187-195. doi: 10.3920/CEP190046

Lacerda, L., Campos, R., Sperb, M., Soares, E., Barbosa, P., Gosinho, E., Ferreira, R., Santos, V., & Gonzalez, F. D. (2006). Hematologic and biochemical parameter in three high-performance horse breeds from southern Brazil. *Archives of Veterinary Science, 11*(2), 40-44. doi: 10.5380/avs.v11i2.6783

MacLeay, J. M. (2010). Disorders of the musculoskeletal system. In S. M. Reed, W. M. Bayly, & D. C. Sellon, *Equine internal medicine* (3nd ed., pp. 1034-1067). St Louis, MO: Saunders Elsevier.

Martin-Gimenez, T., Aguirre-Pascasio, C. N., & De Blas, I. I. (2018). Beyond scoring systems: usefulness of morphometry considering demographic variables, to evaluate neck and overall obesity in Andalusian horses. *Animal, 12*(3), 597-605. doi: 10.1017/S1751731117001628

Martin-Gimenez, T., Blas, I., Aguilera-Tejero, E., Castro, E. D., & Aguirre-Pascasio, C. N. (2016). Endocrine, morphometric, and ultrasonographic characterization of neck adiposity in Andalusian horses. *Domestic Animal Endocrinology, 56,* 57-62. doi: 10.1016/j.domaniend.2016.02.003

Muñoz, L., Ananías, M., Cruces, J., Ortiz, R., & Briones, M. (2019). Body condition in elite Chilean rodeo horses: preliminary study. *Revista de Medicina Veterinaria y Zootecnia, 66*(1), 28-34. doi: 10.15446/rfmvz.v66n1.79389

Siciliano, P. D., Lawrence, L. M., Danielsen, K., Powell, D. M., & Thompson, K. N. (1995). Effect of conditioning and exercise type on serum creatine kinase and aspartate aminotransferase activity. *Equine Veterinary Journal, 27*(18), 243-247. doi: 10.1111/j.2042-3306.1995.tb04929.x

Song, H. J., Oh, S., Quan, S., Ryu, O.-H., Jeong, J.-Y., Hong, K.-H., & Kim, D.-H. (2014). Gender differences in adiponectin levels and body composition in older adults: hallym aging study. *BMC Geriatrics, 14*(8). doi: 10.1186/1471-2318-14-8

Stuchi, L., Valli, C., Stancari, G., Zucca, E., & Ferrucci, F. (2019). Creatine-kinase reference intervals at rest and after maximal exercise in Standardbred racehorses. *Comparative Exercise Physiology, 15*(5), 319-325. doi: 10.3920/CEP190020

Teixeira, A. R., Neto, Ferraz, G. C., Moscardini, A. R. C., Balsamão, G. M., Souza, J. C. F., & Queiroz, A., Neto. (2008). Alterations in muscular enzymes of horses competing

long-distance endurance rides under tropical climate. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60*(3), 543-549. doi: 10.1590/S0102-09352008000300004

Westervelt, R. G., Stouffer, J. R., Hintz, H. F., & Schryver, H. F. (1976). Estimating fatness in horses and ponies. *Journal of Animal Science, 43*(4), 781-785. doi: 10.2527/jas1976.434781x

Wooldridge, A. A., Edwards, H. G., Plaisance, E. P., Applegate, R., Taylor, D. R., Taintor, J., Zhong, O., & Judd, R. L. (2012). Evaluation of high-molecular weight adiponectin in horses. *American Journal of Veterinary Research, 73*(8), 1230-1240. doi: 10.2460/ajvr.73.8.1230

Xu, A., Chan, K. W., Hoo, R. L. C., Wang, Y., Tan, K. C. B., Zhang, J., Chen, B., Lam, M. C., Tse, C., Cooper, G. J. S., & Lam, K. S. L. (2005). Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *The Journal of Biological Chemistry, 280*(18), 18073-18080. doi: 10.1074/jbc.M414231200