Efecto de un protocolo de natación sobre variables hemáticas, metabólicas y cardiovasculares de caballos criollos colombianos obesos

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Contenido

Lista de Abreviaturas

%	Percentage-porcentaje
ATP	Adenosín Tri Fosfato
ATPasa	Enzima que cataliza la descomposición de ATP en ADP y un ion de fosfato libre
CCC	Caballo Criollo Colombiano
CK	Creatinin-Quinasa
CPHs	Colombian Paso horses
CV	Coefficient of Variation
d	day-día
dL	Decilitro
EMS	Equine Metabolic Syndrome
f:	frecuencia respiratoria
FC Máx.	Frecuencia Cardiaca Máxima
FC	Frecuencia Cardiaca
f-SET	Field Standardized Exercise Test
g	gramo
HR	Heart Rate
HRMax.	Maximum Heart Rate
IQR	Inter Quartil Range
K^+	Potasio iónico
kg	kilogramo
L	litro
LPM	Latidos por Minuto
m	metro
ME	Median-mediana
mean Vi	flujo inspiratorio medio o impulso inspiratorio.
min	minuto
ml	mililitro
mm	Milímetros
mmHg	milímetros de mercurio
mmol	Milimoles
Na^+	Sodio iónico
O_2	Oxígeno molecular
pH	Potencial Hidrógeno
rpm	Respiraciones por minuto
SD	Standard Deviation
SME	Síndrome Metabólico Equino
SMH	Síndrome Metabólico Humano
SV	Systolic Volume
te	tiempo espiratorio
ti / ttot	relación entre el tiempo inspiratorio y el tiempo total para el ciclo de respiración
ti	tiempo inspiratorio del ciclo de respiración
VA	ventilación alveolar
VD / VT	relación entre el volumen corriente y espacio muerto
VD	espacio muerto fisiológico
VD	ventilación del espacio muerto
VE / VCO_2	equivalente ventilatorio para la salida de dióxido de carbono
VE / VO_2	ventilador equivalente para la absorción de oxígeno
VE	volumen minuto
VO_2máx	Consumo Máximo de Oxígeno
VT	volumen corriente o tidal
Y	years old

Resumen general

Abstract

Obesity in horses is frequent as a result of nutritional practices and the domestication process that imply a sedentary lifestyle; overweight and obesity in horses are risk factors for the development of disabling diseases such as metabolic syndrome (SME). This condition predisposes animals to experience exercise-induced diseases to a greater extent in contrast to non-obese animals. This work aimed to evaluate the effect of a swimming protocol on hematic, metabolic and cardiovascular variables of obese Colombian Creole horses.

Six (6) Colombian Creole horses were subjected to a swimming protocol with decreasing intensity for four (4) months; the training period was divided into two (2) cycles of two (2) months each. Cycle one (1) and cycle two (2) correspond to training performed at 80% and 70% of maximum heart rate (HR Max), respectively. To evaluate the effect of the training protocol on athletic performance, field standardized exercise tests were performed (f-SET) at the beginning of the study, at the end of cycle 1, and at the end of cycle 2. In each f-SET samples of venous blood for hematological, biochemical, and ion and gas tests. In addition, the subcutaneous fat thickness (SFT) of each horse was measured at the beginning and end of each training cycle, using ultrasound.

A statistical analysis was carried out both to the acute responses found in each f-SET, and to the differences between cycles.

Acute responses to exercise in f-SET were found to be hemoconcentration and negative change in plasma volume, as a result of dehydration. Furthermore, the anaerobic threshold was not similar between the animals. On the other hand, the acute responses in swimming were rapid reaching of the HR max. and higher blood lactate production compared to f-SET. When training adaptations were studied, it was found that the designed swimming training program produced a decrease in resting HR and HR max.

in addition, decreased lactate production both in the f-SET and in swimming. In addition, it was found that the SFT decreased without affecting body weight. There were no changes in the acid-base and electrolyte status, nor were there relevant changes in the hematological or biochemical parameters analyzed.

Conclusions: the swimming training program with decreasing intensity and a duration of four (4) months for untrained Colombian Creole horses produces cardiac and metabolic adaptations that favor aerobic capacity, in addition, it promotes the mobilization of adipose tissue, decreasing the SFT.

Resumen

La obesidad en caballos es frecuente, como resultado de prácticas nutricionales y del proceso de domesticación que implican sedentarismo; el sobrepeso y obesidad en caballos son factores de riesgo para el desarrollo de patologías incapacitantes como el síndrome metabólico (SME). Esta condición predispone a los animales a experimentar enfermedades inducidas por ejercicio en mayor proporción en contraste con animales no obesos. Este trabajo tuvo como objetivo evaluar el efecto de un protocolo de natación sobre variables hemáticas, metabólicas y cardiovasculares de caballos criollos colombianos obesos.

Seis (6) caballos criollos colombianos fueron sometidos a un protocolo de natación con intensidad decreciente durante cuatro (4) meses; el periodo de entrenamiento fue dividido en dos (2) ciclos de dos (2) meses de duración cada uno. El ciclo uno (1) y el ciclo dos (2) correspondían al entrenamiento realizado al 80% y 70% de la frecuencia cardiaca máxima (FC Máx.) respectivamente. Para evaluar el efecto del protocolo de entrenamiento sobre el desempeño atlético se realizaron pruebas de esfuerzo estandarizadas en campo (f-SET) al inicio del estudio, final del ciclo 1, y final del ciclo 2. En cada f-SET se tomaron muestras de sangre venosa para realizar exámenes

hematológicos, bioquímicos y iones y gases. Además, se midió el espesor de tejido adiposo subcutáneo (SFT) de cada caballo al inicio y final de cada ciclo de entrenamiento, utilizando ultrasonido.

Se realizó análisis estadístico tanto a las respuestas agudas encontradas en cada f-SET, como a las diferencias entre ciclos.

Se encontró que las respuestas agudas al ejercicio en la f-SET son hemoconcentración y cambio negativo del volumen plasmático, como resultado de la deshidratación. Además, el umbral anaerobio no fue similar entre los animales. Por su parte, las respuestas agudas en natación fueron alcance rápido de la FC máx. y producción superior de lactato sanguíneo en comparación con el f-SET. Cuando se estudiaron las adaptaciones al entrenamiento se encontró que el programa de natación diseñado produjo, disminución de la FC en reposo y FC máx. además, disminución de la producción de lactato tanto en el f-SET como en natación. Adicionalmente, se comprobó que el SFT disminuyó sin afectar el peso. No se evidenció alteraciones del estado ácido-base y electrolítico, tampoco cambios relevantes de los parámetros hematológicos o bioquímicos analizados.

Conclusiones: el programa de entrenamiento en piscina con intensidad decreciente y una duración de cuatro (4) meses para caballos criollos colombianos desentrenados produce adaptaciones cardiacas y metabólicas que favorecen la capacidad aerobia, además, promueve la movilización del tejido adiposo disminuyendo el SFT.

Introducción General

El sobrepeso y la obesidad en caballos es frecuente, como resultado de prácticas nutricionales y de domesticación, que implican sedentarismo; consecuentemente se le considera, así como en humanos, un asunto de salud pública veterinaria, al constituir un factor de riesgo para el desarrollo de patologías incapacitantes para el equino como el síndrome metabólico (SME) y la laminitis (Johnson et al., 2009).
En los equinos, han sido reportadas frecuencias entre el 45 y 51% de obesidad y problemas metabólicos en el contexto mundial (Johnson et al., 2009). En Colombia existen pocos estudios epidemiológicos del problema en el caballo criollo colombiano (CCC). Rosas (2017) determinó que CCC con condición corporal entre 7 y 9 (según la escala de Henneke) fueron más propensos a padecer resistencia a la insulina, además de evidenciar adiposidad regional y en algunos casos, signos clínicos de laminitis. La tendencia a formar acúmulos de grasa en las áreas del cuello, escápula, base de la cola, prepucio y glándula mamaria y la resistencia a la insulina son comunes en el CCC. Este conjunto de signos es similar y comparable a los que caracterizan el síndrome metabólico de humanos (SMH). Sin embargo, tan solo en 2017 se ha reportado un caso de SME en Colombia (Castillo et al., 2017).

Adicional a los efectos metabólicos y endocrinos, la obesidad en equinos compromete el desempeño atlético, dificulta el éxito del entrenamiento para lograr acondicionamiento físico, y por supuesto predispone al animal a padecer patologías inducidas por el ejercicio (por ejemplo: rabdomiólisis) comparado con animales de condición y composición corporal adecuadas (experiencia clínica de la autora). Por otro lado, la existencia de una condición corporal alta (entre 8 y 9) es el reflejo de un inadecuado manejo nutricional en relación con la disciplina deportiva desempeñada por el caballo.

En consecuencia, se hace necesario el desarrollo de investigación orientada al entrenamiento de CCC, puesto que es común encontrarlos desempeñando actividades deportivas de alto rendimiento, a pesar de encontrarse en condición de obesidad. No se conoce si las adaptaciones fisiológicas al ejercicio, en este caso natación en caballos obesos se producen de forma similar a como sucede en caballos de condición corporal ideal.

El entrenamiento rutinario del CCC es de moderada a muy alta intensidad (FC mayor de 160 lpm), lo que lo constituye en un entrenamiento destinado al desarrollo de la fuerza y de la velocidad durante la ejecución del andar. La intensidad descrita no promueve el desarrollo de la resistencia (capacidad de desempeñarse en su disciplina por largos periodos de tiempo sin experimentar fatiga), y, por otro lado, suele utilizar sustratos energéticos de corta duración como la fosfocreatina, el ATP y la energía resultante de la glicólisis anaerobia utilizando la glucosa y el glucógeno muscular del glucógeno muscular (Boffi, 2008). Por el contrario, el ejercicio aerobio (frecuencia cardiaca entre 100 y 160 lpm) promueve el uso de sustratos energéticos provenientes de la lipólisis, es por esto que el ejercicio para animales obesos debe llevarse a cabo bajo intensidad submáxima (Stich et al., 2000); como caminar y galopar. Todo lo anterior explica la razón por la cual, a pesar de encontrarse bajo alguna rutina de entrenamiento el CCC es propenso a permanecer obeso. Por supuesto, las prácticas nutricionales y alimenticias inadecuadas (sobrecarga de carbohidratos) exacerban esta situación. Adicionalmente, es tradicional que los animales permanezcan en estado de obesidad por razones estéticas culturales.

El CCC se caracteriza por depositar tejido adiposo en regiones específicas del cuerpo como el cuello, la región lumbar, la base de la cola, el prepucio y la ubre, además es frecuente encontrar estos animales en estado de obesidad.

La obesidad y la adiposidad regional en el CCC dificulta el logro de objetivos deportivos claros como el desarrollo de resistencia en la velocidad o de *stamina*, y, por el contrario, hace susceptible al CCC a experimentar fatiga y desórdenes metabólicos que resultan en falla renal y patologías osteomusculares.

La obesidad es el principal factor desencadenante del SME, que a su vez redunda en la presentación de laminitis subclínica crónica (Durham et al., 2019). En consecuencia, el caballo obeso con SME tolera poco cualquier tipo de entrenamiento que se aplique sobre superficies duras como el asfalto, la madera o las pistas con material aglomerado a presión; a pesar de esto el ejercicio es el componente principal en el tratamiento del SME. Entonces, un caballo con laminitis crónica clínica o subclínica solo podrá ejercitarse bajo una carga de trabajo suficiente para promover la movilización de grasa corporal si sus extremidades no golpean el suelo, como es el caso de la natación.

Con el fin de mejorar el desempeño de CCC obesos evitando la superficie dura y el peso del jinete, en Colombia se ha utilizado la natación en forma empírica, de hecho, la construcción de piscinas para caballos se ha popularizado en el país en los últimos cinco años a pesar de que en la mayoría de los casos no están técnicamente concebidas, ni tampoco la prescripción de la natación es hecha por un profesional en el área (experiencia de la autora).

Como experiencia de la autora, se ha utilizado la piscina circular para el desarrollo de resistencia física en caballo criollo colombiano, buscando mejorar el desempeño deportivo en las competencias de cada modalidad de andar de la raza (trote y galope, trocha pura, trocha y galope y paso fino). Debido a que el desempeño atlético se encuentra determinado por múltiples factores, se implementaron mediciones para conocer la respuesta fisiológica de los ejemplares entrenados con natación. Estas mediciones inicialmente obedecieron a la toma de la frecuencia cardiaca en reposo, durante y posterior a la natación, el consumo máximo de oxígeno (VO_2máx) y el depósito de grasa dorsal medido a través de ultrasonido. Los resultados al respecto son anecdóticos y aún no se encuentran reportados en la literatura científica; sin embargo, se conoce que dichas variables permiten establecer cambios orgánicos en respuesta a protocolos de natación modificados quincenalmente. Fue llamativo encontrar ejemplares que ejecutaron la natación controlada 5 días a la semana y evidenciaron al ultrasonido pérdida de tejido adiposo subcutáneo mensual de cerca de 2 mm (espesor). Por supuesto, estos cambios sobre el tejido adiposo sucedieron de manera particular en cada caballo y sugirieron entonces que existen variables que intervienen según las características propias de cada ejemplar. En la experiencia con animales jóvenes (potros a partir del destete), se encontró que la respuesta cardiovascular y de VO_2máx fue positiva, al evidenciar disminución de la frecuencia cardiaca en reposo y aumento del VO_2máx; aunque a simple vista la condición corporal no se encontró afectada, la actividad de la creatinin-quinasa (CK) se elevó notablemente, en otras palabras, la aplicación del ejercicio en piscina para cada animal debe ir acompañado de la evaluación clínica de rutina y de la aplicación de otras variables que sean útiles para monitorear el gasto metabólico.

Conocer los cambios fisiológicos que suceden en un equino obeso, es de gran ayuda en el manejo médico y deportivo de animales que padezcan condiciones restrictivas (por ejemplo: laminitis) debido al depósito excesivo de tejido adiposo.

En el ámbito de la medicina deportiva equina, esta investigación fue un aporte útil en cuanto a protocolos de ejercicio establecidos científicamente, esto en contraste con la poca información actualizada que existe al respecto del uso de la natación en caballos que ejecutan disciplinas mixtas en estado de obesidad, como es el caso del CCC.

Referencias bibliográficas

[1] Johnson P.; Wiedmeyer C.H.; Messer N. 2009. Medical implications of obesity in horse – Lesson for human obesity. Journal of Diabetes Science and Technology. 3(1): 163 -174.

[2] Rosas, M.M. 2017. Diagnóstico de síndrome metabólico equino y su asociación con signos clínicos de laminitis en el caballo criollo colombiano en la sabana de Bogotá. Tesis de grado para optar al título de magíster. Universidad de La Salle. Bogotá.

[3] Castillo, C.; Jaramillo, C; Loaiza, M.J.; Blanco, R. 2017. Equine metabolic syndrome in Colombian creole horse: Case report. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. (BR) 69:1067-1072.

[4] Boffi, F.M. (2008) Entrenamiento y adaptación muscular: sustratos y vías metabólicas para la producción de energía. Rev. Bras. Zootec. 37, 197–201.

[5] Stich, V.; Glisezinski, I.; Berlan, M.; Bulow, J.; Galitzky, J.; Harant, I.; Suljkovicova, H.; Lafontan, M.; Rivie`Re, D.; Crampes, F. 2000. Adipose tissue lipolysis is increased during a repeated bout of aerobic exercise. Journal of applied physiology. (USA). 88: 1277–1283.

[6] Durham, A.E.; Frank, N.; McGowan, C.M.; Menzies-Gow, N.J.; Roelfsema, E.; Vervuert, I.; Feige, K.; Fey, K. 2019. ECEIM consensus statement on equine metabolic syndrome. J. Vet. Intern. Med. 33, 335–349.

Objetivos

Objetivo General

Evaluar el efecto de un protocolo de natación sobre variables hemáticas, metabólicas y cardiovasculares de caballos criollos colombianos (CCC) obesos.

Objetivos Específicos

1. Describir las respuestas hemáticas, metabólicas y cardiovasculares al ejercicio en piscina en un grupo de CCC obesos.
2. Describir las adaptaciones hemáticas y metabólicas alcanzadas por un grupo de CCC obesos sometidos a un protocolo de natación.
3. Identificar las adaptaciones cardiovasculares alcanzadas por un grupo de CCC obesos sometidos a un protocolo de natación.
4. Evaluar los cambios producidos sobre el espesor del tejido adiposo de un grupo de CCC sometidos a un protocolo de natación.

Marco Teórico

Fisiología del ejercicio en el caballo

Aparato musculoesquelético

Dependiendo de la naturaleza (tipo, frecuencia, intensidad y duración) y del estímulo (ejercicio o entrenamiento), la repuesta adaptativa puede tomar diferentes formas: hipertrofia, remodelación sin hipertrofia (p.e. cambios bioquímicos y vascularización) y respuesta mixta (figura 1). Usualmente sucede la remodelación fenotípica sin hipertrofia durante un periodo de entrenamiento prolongado, durante esta adaptación la fibra sufre una reorganización profunda que involucra activación y supresión de genes de forma secuencial y selectiva (Hinchcliff et al., 2008).

Los cambios más evidentes suceden en músculos con mayor proporción de fibras de contracción rápida, las cuales son más glucolíticas con el curso del entrenamiento, en contraste con las fibras de contracción lenta que no demuestran cambios evidentes a pesar de los estímulos físicos producidos por el ejercicio. El entrenamiento de alta intensidad y corta duración suele provocar hipertrofia, no se ha demostrado la hiperplasia del músculo por aumento en el número de fibras musculares como sucede en humanos (Hinchcliff et al., 2008).

La adaptación muscular más rápida y común al entrenamiento es el incremento de la actividad enzimática del metabolismo aerobio. Estos cambios están asociados al incremento de mitocondrias y densidad capilar. Las respuestas adaptativas más tardías involucran mejoría en la difusión de O_2 y remoción de residuos metabólicos. Una adaptación importante del músculo durante el entrenamiento submáximo es el aumento de la bomba Na^+ K^+ - ATPasa junto a la disminución en la salida de K^+ (Hinchcliff et al., 2008).

Figura 1. Resumen de las tres respuestas básicas del músculo esquelético al entrenamiento: (1) remodelación con hipertrofia, (2) remodelación sin hipertrofia, y (3) mixta (remodelación con hipertrofia). Posibles estímulos y la naturaleza de las respuestas fisiológicas. Adaptado de Hinchcliff et al. (2008).

Sistema respiratorio

La ventilación es un proceso fisiológico determinante durante el ejercicio en el caballo. A partir de la ventilación adecuada sucede la perfusión y el intercambio gaseoso correcto hacia los tejidos, por lo tanto, de la adecuada ventilación depende el consumo de oxígeno (VO_2) (Klein, 2014). Las variables ventilatorias valoradas durante el ejercicio se resumen en la Tabla 1.

Valor	Reposo	Caminando	Galope	Recuperación
$\dot{V}O_2$ máx (%)	3,3	14	100	20
VT (L)	5,6	5,8	13,2	6,5
VD (L)	3,4	3,4	2,6	3,5
VA (L)	2,2	2,4	10,6	3
VD/VT (%)	60	58	20	54
FR (rpm)	14	65	121	110
t_i (s)	1,9	0,45	0,25	0,29
t_e (s)	2,4	0,47	0,25	0,26
t_i/t_{tot} (%)	44	49	50	53
$\dot{V}_E$ (L/m)	78	377	1598	715
$\dot{V}_D$ (L/m)	47	219	320	386
$\dot{V}_A$ (L/m)	31	158	1278	329
$\dot{V}_E/\dot{V}O_2$ (L/L)	35	40	24	55
$\dot{V}_E/\dot{V}CO_2$ (L/L)	43	48	23	51
$\dot{V}_i$ promedio (L/s)	2,9	13	53	26

Tabla 1. Valores respiratorios promedio en caballos pura sangre de carreras sanos, edad promedio 5 años y peso promedio 470 kg, corriendo en banda rodante. Adaptado de Hodgson y Rose (1994).

VO_2máx: porcentaje de consumo máximo de oxígeno; VT: volumen corriente; VD: espacio muerto fisiológico; VA: volumen alveolar; VD / VT: relación entre el volumen corriente y espacio muerto; FR: frecuencia respiratoria; ti: tiempo inspiratorio del ciclo de respiración; te: tiempo espiratorio; ti / ttot: relación entre el tiempo inspiratorio y el tiempo total para el ciclo de respiración; VE: volumen minuto; VD: ventilación del espacio muerto; VA: ventilación alveolar; VE / VO2: ventilador equivalente para la absorción de oxígeno; VE / VCO2: equivalente ventilatorio para la salida de dióxido de carbono; Vi promedio: flujo inspiratorio medio o impulso inspiratorio.

En los caballos al galope, la respiración y la locomoción están obligatoriamente acoplados. Las frecuencias de paso y respiración reportadas promedian 110 a 130 por minuto con valores máximos de 148 por minuto. Por lo tanto, cuando el caballo galopa, el aumento en la ventilación minuto con velocidad creciente se debe principalmente al aumento en el volumen corriente más que en la frecuencia respiratoria (Boffi, 2007; Hinchcliff et al., 2008).

Durante el ejercicio, ajustes fisiológicos como dilatación de las narinas, abducción completa de la laringe, y la broncodilatación tiende a facilitar el aumento del flujo de aire y disminuye la resistencia al aumentar el área transversal de las vías respiratorias y, por lo tanto, su radio (Jones et al., 2020).

Sin embargo, a pesar de estas modificaciones físicas inducidas por el ejercicio, el ejercicio intenso induce el aumento en más del doble de la resistencia pulmonar como resultado de factores físicos como fricciones, turbulencias, distribución no homogénea del flujo a lo largo de las vías respiratorias y los alvéolos, y las vías aéreas transversales (Hodgson y Rose, 1994).

Durante el ejercicio submáximo la tensión de gases sanguíneos no cambia sustancialmente, sin embargo, el aumento de la frecuencia respiratoria y volúmenes ventilatorios responden a mecanismos termorregulatorios y de gasto cardiaco. Por otro lado, durante el ejercicio intenso la disminución de la presión parcial de oxígeno arterial y el pH sanguíneo desencadena hiperventilación, excepto en presencia de fatiga muscular respiratoria (Hinchcliff et al., 2008).

Sistema cardiovascular

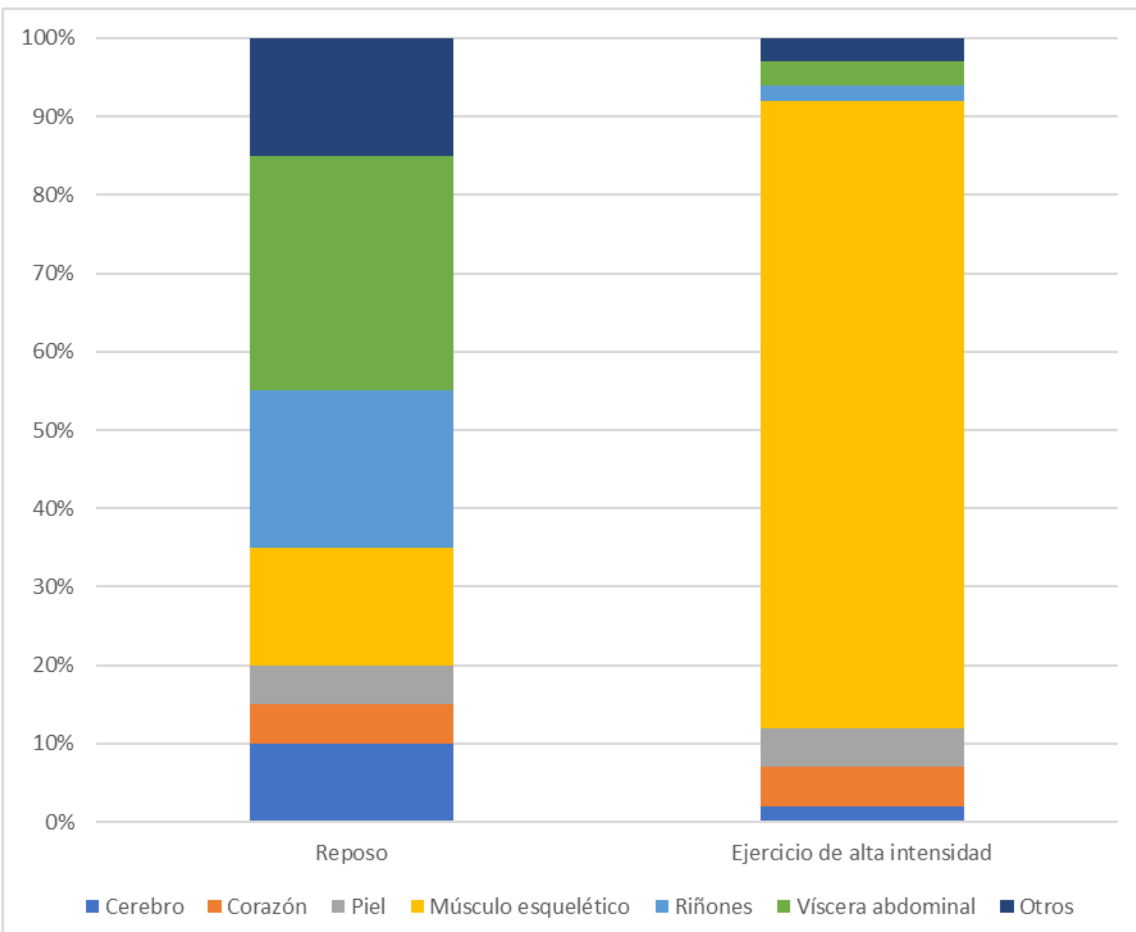

Figura 2. Distribución del gasto cardíaco en reposo y durante el ejercicio de alta intensidad. En el caballo pura sangre de carreras altamente capacitado y en buena forma, es posible que el flujo sanguíneo del músculo esquelético pueda alcanzar hasta el 90% del gasto cardíaco. Adaptado de Hinchcliff et al. (2008).

La respuesta más evidente del sistema cardiovascular al ejercicio es la resultante del aumento del tono simpático, con la consecuente modificación del gasto cardiaco en función de la frecuencia cardiaca. Como se puede notar en la figura 3, el gasto cardiaco durante el ejercicio es dirigido en un 80-90% al músculo esquelético, restringiendo a varios sistemas orgánicos del suministro de oxígeno. El gasto cardíaco es el medio más importante para aumentar el suministro de O_2 a los músculos durante el ejercicio y es el principal determinante del consumo de oxígeno (VO_2) que puede variar de 90 hasta 220 ml / kg / min (Hinchcliff et al., 2008).

Variable	Reposo	Ejercicio
Frecuencia cardiaca (latidos por minuto)	30	210-250
Gasto cardiaco (Litros por minuto)	30	240-450
Presión arterial sistólica/diastólica (milímetros de mercurio)	130/80	230/110
Consumo de oxígeno (mililitros por minuto por kilogramo)	2-4	160-220
Diferencia de oxígeno arterio-venoso (mililitros por cada 100 mililitros de sangre)	5	20-25

Tabla 2. Respuestas cardiovasculares al ejercicio máximo en un caballo de 500 kg. Adaptado de (Hinchcliff et al., 2008).

Como puede verse en la tabla 2, durante el ejercicio las variables hemodinámicas se incrementan de manera notoria. Todas ellas pueden modificarse en función del entrenamiento y las adaptaciones fisiológicas al ejercicio como la hipertrofia cardiaca (Hinchcliff et al., 2008).

Referencias bibliográficas

[1] Boffi, F. M. Fisiología del ejercicio en equinos, Buenos Aires: Intermédica, 2007.

[2] Hinchcliff, K. W.; Geor, R. J.; Kaneps, A. J. Equine exercise physiology: the science in the athletic horse, Saunders Elsevier, 2008.

[3] Hodgson, D. R.; Rose, R. J. Principles and practice of equine sports medicine: the athletic horse, Philadelphia: Saunders, 1994.

[4] Jones, S., Franklin, S., Martin, C. and Steel, C. (2020) Complete upper airway collapse and apnoea during tethered swimming in horses. Equine Vet. J. 52, 352–358.

[5] Klein, B. G. Fisiología veterinaria de Cuningham, Barcelona: Elsevier Saunders, 2014.

Respuesta hemática y metabólica al ejercicio

Otros cambios determinantes en el desempeño óptimo son las adaptaciones hemáticas e inmunológicas al ejercicio. Como se puede ver en la figura 3, los cambios en el leucograma son notorios como efecto agudo del ejercicio, la inadaptación del caballo al ejercicio puede ser deletéreo para el funcionamiento celular del sistema inmune.

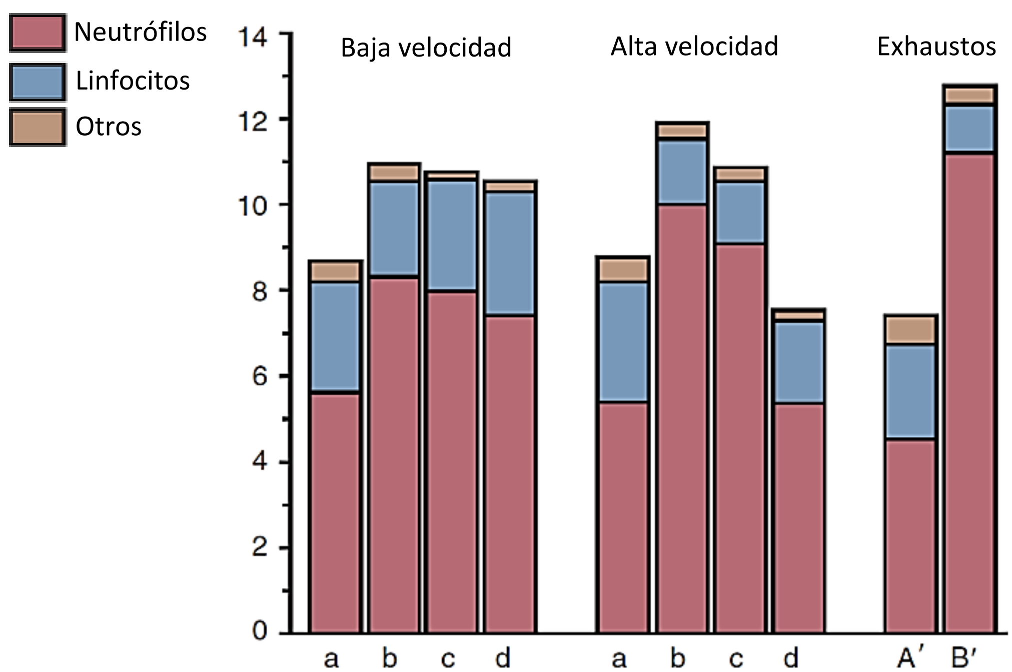

Figura 3. Caballos de enduro: a= antes de la carrera; b= inmediatamente después de la carrera; c= 30 minutos después de la carrera; d= 24 horas después de la carrera.

Caballos de enduro exhaustos= A' =reposo; B '= caballos exhaustos, después de la carrera. Adaptado de (Hodgson y Rose, 1994).

Los cambios hemáticos encontrados en animales que practican ejercicio intenso corresponden al aumento del hematocrito, en cambio aquellos que se ejercitan a intensidad submáxima se encuentra disminuido este parámetro por causa de la expansión de volumen plasmático. Algunos pueden experimentar hemólisis por la fragilidad que adquiere el eritrocito debido al cambio de pH (Hodgson y Rose, 1994).

Efectos metabólicos de la actividad endocrina durante el ejercicio en caballos

Los efectos fisiológicos logrados por la actividad hormonal durante el ejercicio en el caballo se dividen en dos grupos 1) control de la volemia y el gasto cardiaco y 2) movilización y utilización de los recursos energéticos. Sin embargo, también se conocen otros efectos como los producidos por los opioides endógenos durante el ejercicio intenso o los esteroides sexuales.

Respuesta endocrina de origen hipotalámico-hipofisiario:

Las acciones fisiológicas de las hormonas hipotalámico-hipofisiarias reducen los límites para que el animal pueda seguir con el ejercicio prolongado y extenuante al condicionar la actitud mental al aprendizaje durante el entrenamiento y la percepción de los esfuerzos y el dolor muscular durante la actividad física (Boffi, 2007).

Ha sido comprobado que la producción de opioides endógenos como las β-endorfinas y la producción de hormona adenocorticotropa (ACTH) se relacionan con la intensidad del ejercicio en caballos, específicamente en caballos de salto (Cravana et al., 2017). Dichos caballos mostraron que los opioides son producidos en mayor proporción cuando el ejercicio se torna más intenso al igual que la ACTH, vasopresina u hormona antidiurética (ADH); al parecer esto sucede como mecanismo de protección corporal contra el dolor producido por la actividad muscular y el estrés producido por el reto impuesto al caballo. Este mecanismo se ha encontrado en caballos con alto desempeño atlético y parece mejorar su rendimiento (Boffi, 2007).

El efecto analgésico de las β-endorfinas producidas durante el ejercicio es dependiente de su producción y esta a su vez está relacionada con un umbral alcanzado en el ejercicio intenso (aproximadamente 60% del consumo máximo de oxígeno - VO2 máx-). Al parecer el aumento en la producción de β-endorfinas coincide con el momento en el cual se nota un incremento en las catecolaminas, renina y lactato. Esto supone que el requerimiento de estos opioides endógenos incrementa en la medida en que aumenta el metabolismo anaerobio (Hinchcliff et al., 2008).

La actividad de las β-endorfinas producidas en las *pars intermedia* y *pars distalis* de la hipófisis, ejercen efectos sobre las gonadotropinas, por lo tanto, también "regulan" la actividad reproductiva de animales de alto rendimiento. Esto podría explicar parcialmente la razón por la cual animales de alto rendimiento sometidos a ejercicio intenso pueden experimentar alteraciones reproductivas.

La producción de ADH está mayormente relacionada con el aumento de la osmolaridad plasmática como consecuencia de la deshidratación inducida por el ejercicio, en búsqueda de recuperar una concentración de solutos plasmáticos totales y evitar la pérdida de agua. Sin embargo, cuando se trata de la respuesta aguda al ejercicio la secreción de ADH sucede por debajo del umbral que produce sus efectos antidiuréticos, también conocido como efecto extrarrenal que consiste en generar vasoconstricción y evitar secuestro hemático en el bazo mientras se realiza ejercicio (Hinchcliff et al., 2008).

La somatotropina no ha demostrado secretarse regulada bajo algún patrón específico como respuesta al ejercicio en caballos, su suministro exógeno parece mejorar condiciones físicas relacionadas con el volumen muscular o el proceso de mineralización ósea, sin embargo, no se ha relacionado con mejoría del desempeño. Su medición no es considerada de interés en el análisis del desempeño atlético del caballo (Hinchcliff et al., 2008).

Es importante tener en cuenta la conexión que existe entre el funcionamiento nervioso y la secreción hormonal como sucede en el caso de hormonas secretadas por la neurohipófisis (Hinchcliff et al., 2008); dicha síntesis y secreción hipotalámica está dada por el estímulo de aferentes nerviosos que llevan a su liberación (Klein, 2014), lo que significa que situaciones medioambientales que se puedan desarrollar durante el ejercicio o entrenamiento que produzcan alerta o interés sexual puede alterar la respuesta fisiológica.

Respuesta endocrina de origen tiroideo

El comportamiento endocrino más complejo durante el ejercicio en el caballo es el de las hormonas tiroideas, debido a la alta cantidad de factores que pueden afectar su secreción y uso activo en los tejidos, de hecho, algunas características como sexo y edad deben ser tenidas en cuenta al momento de interpretar los resultados de dichas hormonas durante el ejercicio.

El aumento de la liberación de la hormona estimulante de la tiroides (TSH) parece relacionarse con la intensidad del ejercicio, sin embargo, parece mantenerse cerca de un umbral todavía no establecido en caballos, y consecuentemente se le ha relacionado con la movilización de sustratos energéticos con el fin de evitar la fatiga central (Hinchcliff et al., 2008).

Es claro, que los animales poco entrenados evidencian aumentos sensibles de la triyodotironina (T3) durante los periodos inmediatamente posteriores al ejercicio, mientras que en animales experimentados la concentración plasmática de todas las hormonas tiroideas medibles permanece en valores fisiológicos, demostrando adaptación al entrenamiento. Al parecer, las hormonas tiroideas son producidas en mayor cantidad durante el ejercicio sin embargo su activación y utilización tisular rápida (facilitada por el aumento en la vascularización del tejido musculoesquelético principalmente) estimulan la producción glandular de más hormonas que mantienen los valores fisiológicos esperados en sangre (Boffi, 2007). Por lo tanto, se le relaciona con requerimientos de movilización energética durante la actividad física.

Otra hormona producida en la glándula tiroides es la calcitonina. Aunque su dinámica durante el ejercicio no aparece como protagónica, se ha demostrado que durante el entrenamiento crónico de caballos de carreras se le encuentra aumentada relacionada con la neoformación de hueso y el aumento de la densidad ósea. Es posible que su actuación durante el ejercicio repetido sea el de responder a las adaptaciones biomecánicas logradas por el hueso (Hinchcliff et al., 2008).

Estudios referentes a la actividad de la paratohormona (PTH) durante el ejercicio en caballos de enduro y salto han demostrado que la hipocalcemia inducida por el consumo metabólico promueve el aumento de la concentración plasmática de PTH en función de mantener la cantidad suficiente de calcio circulante durante la actividad física. Es importante tener presente que existen otros factores implicados en la hipocalcemia que pueden asociarse a la respuesta de la PTH como es la hiperalbuminemia y los cambios de pH sanguíneo que afectan la relación calcio-proteínas (Aguilera-Tejero et al., 1998; Aguilera-Tejero et al.,2001).

Respuesta endocrina de origen adrenal

Con respecto de la producción y liberación de catecolaminas durante el ejercicio es factible decir que se caracteriza por ser la respuesta clásica de estrés. A pesar de que su liberación durante el ejercicio puede suceder constantemente, los cambios en la concentración plasmática no se hacen muy evidentes hasta tanto el caballo no experimente al menos el 50% de su capacidad aerobia. Como es conocido las catecolaminas liberadas producen efectos cardiacos como inotropismo y cronotropismo, además de mejorar el gasto cardiaco. Adicionalmente, la espleno-contracción es el resultado del estímulo de estas sustancias sobre el bazo durante el ejercicio.

Por otro lado, la estimulación de las catecolaminas sobre los receptores β2 adrenérgicos ubicados en las vías respiratorias producen broncodilatación facilitando de esta forma la ventilación. Por supuesto es claro el efecto hiperglicemiante de las catecolaminas vía movilización de glucógeno. Aunque todos estos efectos son factibles de evidenciar durante el ejercicio es más una respuesta de estrés clásico que una respuesta puramente ocasionada por el ejercicio.

Efectos propios del ejercicio relacionados con la producción y secreción de catecolaminas son aquellos obtenidos a partir del entrenamiento, puesto que ha sido demostrado que la sensibilidad y densidad de receptores adrenérgicos cambian en diferentes tejidos, por ejemplo: receptores α adrenérgicos en el miocardio disminuyen, mientras que los receptores β adrenérgicos aumentan en músculo esquelético y tejido de las vías respiratorias (Hinchcliff et al., 2008), ocasionando disminución de la frecuencia cardiaca (posiblemente acompañada de adaptaciones morfológicas cardiacas como la hipertrofia) y aumento de la capacidad de ventilación respectivamente.

Por otro lado, la producción de mineralocorticoides y glucocorticoides como la aldosterona y el cortisol respectivamente participan en la homeostasis durante el ejercicio de diversas maneras.

La aldosterona es producida en mayor medida durante el ejercicio cuando el efecto de este es la hiperkalemia, al tratarse de la hormona encargada de estimular la excreción de potasio, sin embargo, cuando sucede este efecto no son anulados los demás efectos conocidos por el SRAA como activar la reabsorción renal de sodio y cloro, razón por la cual se le puede encontrar a ambos cerca a sus valores normales a pesar de encontrar hiperkalemia.

El cortisol secretado durante el ejercicio puede obedecer a la respuesta clásica de estrés actuando como hiperglicemiante con prioridad hacia el sistema nervioso. Sin embargo, no todos los efectos durante el ejercicio se restringen a la movilización de sustratos energéticos, en ocasiones la secreción continua de cortisol durante el ejercicio intenso estimula la regeneración tisular como sucede en el músculo esquelético, de hecho, se le ha adjudicado la propiedad de actuar como antiinflamatorio durante procesos de daño que pueden originarse a partir del entrenamiento extenuante (Hinchcliff et al., 2008).

Se ha sugerido que el entrenamiento causa una disminución más rápida de los niveles de cortisol haciendo que estos regresen a los valores de reposo. En efecto, en caballos sin experiencia que realizan saltos de exhibición se registró una disminución más lenta que en caballos con más experiencia (Boffi, 2007).

Respuesta endocrina de origen digestivo

Las hormonas pancreáticas responden al ejercicio agudo con su disminución, relacionado con el efecto directo del cortisol sobre estas. Sin embargo, se ha demostrado que la relación glucosa-insulina se hace estrecha cuando el animal ha sido sometido a entrenamiento de resistencia (Boffi, 2007). La insulina no es utilizada propiamente durante el ejercicio para la internalización de la glucosa, de hecho, durante el esfuerzo físico la gluneogenésis es inhibida para garantizar que la glucosa circulante no se almacene, sino que por el contrario sea utilizada por los tejidos. Durante el ejercicio es más activa la glucogenólisis y este evento genera depleción de las reservas energéticas que no se repondrán durante el ejercicio, es entonces cuando la insulina pasa a ser utilizada durante el descanso y la recuperación para restituir el glucógeno depletado (Hinchcliff et al., 2008). Por el contrario, el glucagón aparece como la hormona más importante de la movilización de sustratos energéticos durante el ejercicio, aunque su producción depende de la intensidad del ejercicio ha sido demostrada su participación en disciplinas de largo aliento principalmente (Hinchcliff et al., 2008).

El polipéptido intestinal vasoactivo está relacionado con la necesidad corporal de movilizar sustratos energéticos mediante lipólisis y glucogenólisis hepática, por lo tanto, se le encuentra aumentado durante el ejercicio de largo aliento (Hinchcliff et al., 2008). Un evento contrario sucede durante el ejercicio que parece ser promotor del desarrollo de úlceras gástricas: la hipergastrinemia, de la cual se sabe poco como mecanismo activo durante la actividad física puesto que su actividad principal es la estimulación de la producción de ácido clorhídrico (Zuluaga et al., 2018).

Con relación a la leptina, durante el ejercicio no se ha encontrado un funcionamiento específico sin embargo los niveles encontrados en caballos obesos que no responden al mecanismo de gasto energético e inhibición del apetito funcionan durante el entrenamiento para valorar el balance energético del animal.

Otras hormonas digestivas como la somatostatina, polipéptido pancreático y amilina parecen responder al ejercicio conforme se espera según la respuesta simpática, con la reducción en la producción de estas y por ende en la actividad digestiva (Boffi, 2007; Hinchcliff et al., 2008).

Respuesta endocrina de origen renal y cardiaco

Quizá uno de los eventos endocrinos relacionados con el ejercicio más evidente y responsivo es la estimulación del sistema renina angiotensina aldosterona (SRAA). Su activación puede responder a eventos mecánicos sobre el volumen vascular, la presión de oxígeno arterial (pO_2) e incluso la disminución en la concentración plasmática de cloro y sodio; todos ellos susceptibles de ser modificados durante el ejercicio a causa de la pérdida de iones en la sudoración y la pérdida de agua durante la termorregulación, además del consumo constante de oxígeno (Klein, 2014). Es el aparato yuxtaglomerular el encargado de percibir los cambios en dichas variables para producir la renina, que a su vez se encargará de metabolizar el angiotensinógeno a angiotensina I y de allí en adelante promover vasoconstricción, retención de agua y sodio y excreción de potasio entre otros. Con relación a la eritropoyetina no se ha demostrado su participación activa durante el ejercicio, sin embargo algunos investigadores han intentado establecer los efectos encontrados en caballos ejercitados en banda rodante que fueron suplementados exógenamente con eritropoyetina recombinante humana; se encontró que aumenta la capacidad aerobia de dichos animales, sin embargo existe evidencia en humanos de la generación de retroalimentación negativa que induce la disminución de la eritropoyetina endógena (McKeever et al., 2006).

La vaso actividad durante el ejercicio como se ha descrito está ligada a diversas respuestas endocrinas. La producción de endotelinas sin embargo no es protagonista, aunque se pensara que la vasoconstricción producida por ellas puede ser responsable del incremento de la presión arterial. Se ha demostrado que su producción es importante durante la recuperación momento en el cual puede existir hipovolemia por deshidratación y son las endotelinas las encargadas de aportar en el restablecimiento de la presión sanguínea.

Durante el ejercicio máximo la presión venosa aumenta considerablemente, generando el estímulo suficiente para que el atrio produzca péptidos natriuréticos capaces de generar vasodilatación y natriuresis. Este efecto es más potente que la acción del SRAA (Boffi, 2007).

Respuesta endocrina de origen reproductivo

Testosterona y estrógenos. Los primeros producidos también en la corteza adrenal producen aumento del depósito proteico en los tejidos como en el caso del músculo esquelético. Por otro lado, los estrógenos inducen la reposición del glucógeno muscular por lo tanto participa en el proceso de recuperación pos-ejercicio (Boffi, 2007; Zuluaga et al., 2018)

Referencias bibliográficas

[1] Hodgson, D. R.; Rose, R. J. Principles and practice of equine sports medicine: the athletic horse, Philadelphia: Saunders, 1994.

[2] Boffi, F. M. Fisiología del ejercicio en equinos, Buenos Aires: Intermédica, 2007.

[3] Cravana, C.; Medica, P.; Ragonese G.; Fazio, E. Influence of training and competitive sessions on peripheral. 2017. Veterinary World. 10, 1: 67-73.

[4] Hinchcliff, K. W.; Geor, R. J.; Kaneps, A. J. Equine exercise physiology: the science in the athletic horse, Saunders Elsevier, 2008.

[5] Klein, B. G. Fisiología veterinaria de Cuningham, Barcelona: Elsevier Saunders, 2014.

[6] Aguilera-Tejero, E; Estepa, J. C.; López, I.; Bas, S.; Garfia, B.; Rodríguez, M. Plasma ionized calcium and parathyroid hormone concentrations in horses after endurance rides. 2001. Journal of the american veterinary medical association. 219 (4): 488-490.

[7] Aguilera-Tejero, E.; Garfia, B.; Estepa, J.; López, I.; Mayer-Valor, R.; Rodríguez, M. Effects of exercise and EDTA administration on blood ionized calcium and parathyroid hormone in horses. 1998. American journal of veterinary research. 59 (12).

[8] Zuluaga, A. M.; Ramirez, N. F.; Martinez, J. R. Equine gastric ulcerative syndrome in Antioquia (Colombia): Frequency and risk factors. 2018. Revista Colombiana de Ciencias Pecuarias. 31 (2):139-149.

[9] McKeever, K. H.; Agans, J. M.; Geiser, S.; Lorimer, P. J.; Maylin, G. A. Low dose exogenous erythropoietin elicits an ergogenic effect in Standardbred horses. 2006. Equine exercise physiology. 36: 233-238.

Acidosis metabólica inducida por el ejercicio

La acidosis metabólica ha sido determinada tradicionalmente bajo la premisa del cambio en las variables bicarbonato (HCO_3^-), concentración plasmática de CO_2 (PCO_2) y pH (aproximación de Henderson-Hasselbach). Sin embargo, se ha demostrado que existen otros factores destacados que participan en las alteraciones del pH corporal, como son los ácidos débiles, los iones fuertes inorgánicos y orgánicos (p.e. lactato) (aproximación de Stewart) (Hinchcliff et al., 2008).

Durante el ejercicio la producción de lactato y de protones asociados disminuye el pH, la respuesta fisiológica constituye en primera instancia la producción y liberación de HCO_3^- que a su vez es el responsable de la producción no metabólica de CO_2 que deberá ser eliminado del organismo a través de la ventilación (hiperventilación). A pesar de que esta teoría es aceptada y aplicada en la clínica deportiva, no es la única explicación; al examinar la bioquímica de la glicólisis se puede encontrar que durante la respiración celular que sucede en la mitocondria se producen protones durante la fosforilación del ATP, es decir, que la acidosis metabólica no sucederá únicamente durante el ejercicio de alta intensidad sino que puede presentarse durante el ejercicio moderado siempre que los mecanismos de compensación del pH no sean suficientes (Robergs et al., 2004).

En el caballo en ejercicio, todo el balance ácido-base del organismo depende de las respuestas integradas entre los sistemas muscular, cardio-respiratorio, digestivo, tegumentario y renal. De todos ellos el sistema músculo esquelético es el mayor productor de ácidos y subproductos metabólicos derivados del ejercicio, principalmente del ejercicio de alta y máxima intensidad. Sin embargo, otros sistemas pueden modificar la proporción de ácidos débiles o iones fuertes como el sodio (Na^+), potasio (K^+) y Cloro (Cl^-), por ejemplo, a través de la sudoración o el consumo metabólico de los mismos (Hinchcliff et al., 2008).

La producción de protones (H^+) durante el ejercicio es muy activa, durante la glicólisis anaerobia se pueden producir cerca de 0.425 mol de H^+ por cada mol de lactato que se produzca. Estos protones son altamente reactivos y pueden formar fácilmente otros compuestos que se encargarán de modificar el pH orgánico.

$$ATP + H_2O \rightarrow ADP + Pi + 0.425\ H^+$$

El examen de la secuencia de reacciones bioquímicas de la producción de energía demuestra que los protones producidos por la hidrólisis de ATP está más que contrarrestados por los consumidos por la hidrólisis de creatina fosfato (PCr_2^-), lo que resulta en una disminución bien conocida en protones intracelular al inicio del ejercicio. Dentro de los primeros minutos de ejercicio ocurre una disminución de la dependencia de la hidrólisis de PCr_2^- para regenerar ATP, ya que el ATP se produce cada vez más a partir de la glucogenólisis / glucólisis, así como de fuentes aeróbicas. Cuando el ejercicio continúa por encima del umbral de lactato, la reacción combinada de hidrólisis de ATP y producción de lactato produce cantidades casi equivalentes de lactato y H^+

dentro del músculo esquelético. Usualmente se produce acidosis (Hinchcliff et al., 2008).

Cabe mencionar que el ejercicio de alta y máxima intensidad aumenta el gasto cardiaco en función de la frecuencia cardiaca, la presión arterial media y la viscosidad sanguínea, restringiendo la entrega de oxígeno a los tejidos y promoviendo la necesidad de producir energía (ATP) a través de vías metabólicas anaerobias (≥80% del VO_2máx) (Boffi, 2007).

La disminución del pH corporal produce efectos sobre la musculatura esquelética como:

- Disminución de la actividad glucolítica y glucogenolítica → disminución en la producción de ATP → fatiga
- Disminución del calcio en el retículo sarcoplásmico → aumento del calcio citosólico →disminución de la actividad de la miosina-ATPasa→ disminución del acople actina-miosina →disminución de la tasa de contracción muscular →fatiga
- Inhibición de la unión del calcio a la troponina C →disminución en los acoples actina-miosina →disminución de la fuerza de contracción →fatiga
- Disminución en el aclaramiento del lactato muscular →acidificación intracelular prolongada debido a la retención de aniones ácidos fuertes

Sobre la base de la bioquímica, los protones realizan un cambio en el flujo a medida que el ejercicio progresa del estado-estable al estado no-estable. Para el equilibrio celular la hidrólisis de ATP es requerida para alimentar el trabajo de las células, como en la contracción muscular. Esta es claramente la principal fuente de liberación de protones en la contracción del músculo esquelético, y cuando el NADH y los protones de las reacciones citosólicas se producen a tasas superiores a la capacidad mitocondrial, la producción de lactato ayuda a la reducción citosólica, que esencialmente explica la liberación de protones por la glucólisis. Sin embargo, como la tasa de hidrólisis de ATP excede a todas las demás reacciones, la tasa de liberación de protones eventualmente excede la amortiguación metabólica de protones por la producción de lactato y la descomposición del fosfato de creatina, así como la amortiguación de protones por fosfatos inorgánicos (Pi), aminoácidos y proteínas. Además, una vez que se excede la capacidad máxima de eliminación de lactato / protón de la célula, se produce la acumulación de protones (disminución del pH celular) (Robergs et al., 2004). La figura 4 resume estos eventos durante el ejercicio de alta intensidad. La figura 5 muestra los cambios en los mecanismos compensatorios de la acidificación intracelular durante la transición de la intensidad del ejercicio.

La acidosis intracelular sucede posteriormente a la acidosis metabólica, puesto que como mecanismo de neutralización del pH sanguíneo se libera K^+ al intersticio y vasculatura para internalizar los protones dentro de la célula, pero este efecto genera hiperkalemia.

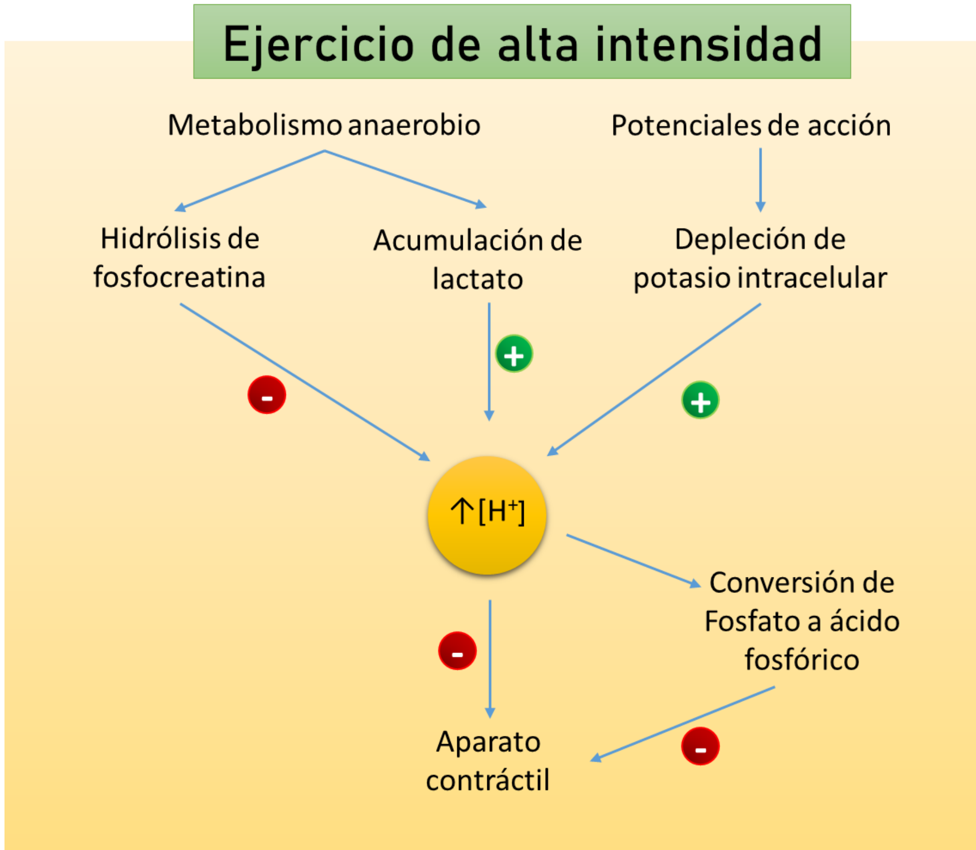

Figura 4. Resumen de eventos que contribuyen a la acidosis intracelular y la fatiga del músculo esquelético durante el ejercicio de alta intensidad. (-) indica una disminución en la acumulación de funciones, mientras que (+) indica una contribución positiva al aumento de [H +]. Adaptado de (Hinchcliff et al., 2008).

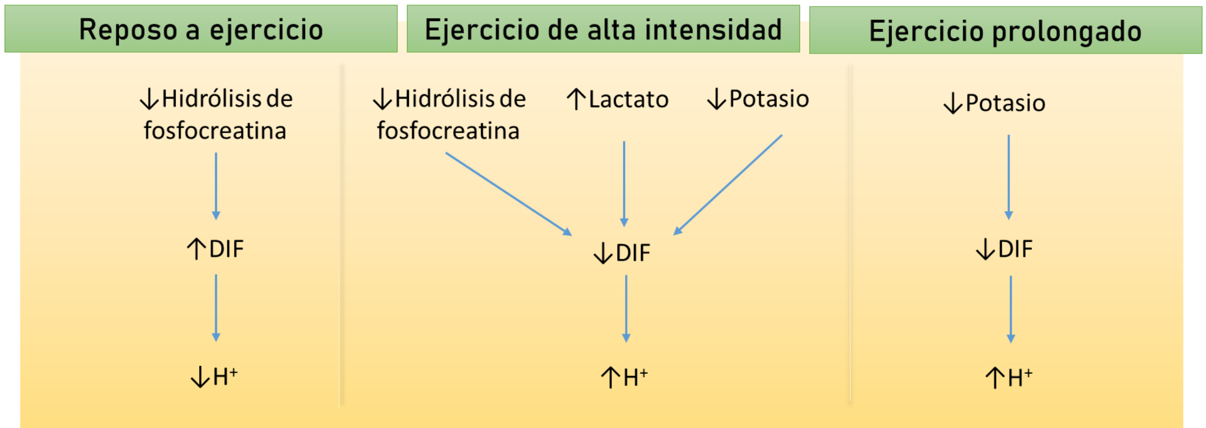

Figura 5. Factores que afectan la diferencia de iones fuertes (DIF) muscular, y el impacto sobre la concentración de protones muscular durante el ejercicio. Adaptado de (Hinchcliff et al., 2008).

Otro mecanismo involucrado en la acidosis metabólica inducida por ejercicio es la pérdida de Cl^-. El cloro es requerido en el eritrocito para realizar la liberación a la luz vascular de HCO_3^-, que actuará como uno de los principales buffers durante la generación de protones, sin embargo, cuando su liberación no es suficiente por causa de la hipocloremia, se denomina acidosis metabólica hipoclorémica (figura 5).

Para finalizar, cabe anotar que los cambios en el pH afectan la capacidad de asociación del oxígeno con la hemoglobina y participar en la fatiga a partir de la hipoxia tisular.

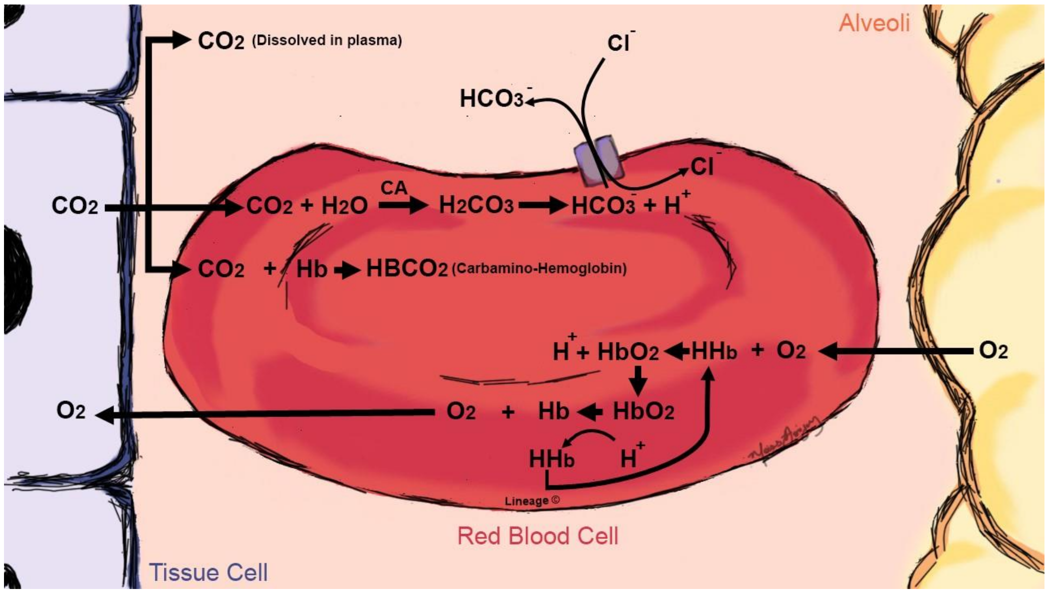

Figura 6. Reacciones bioquímicas al interior del eritrocito relacionadas con la síntesis y liberación de bicarbonato. Tomado de (Dominguez, 2019).

Referencias bibliográficas

[1] K. W. Hinchcliff, R. J. Geor y A. J. Kaneps, Equine exercise physiology: the science in the athletic horse, Saunders Elsevier, 2008.

[2] R. A. Robergs, F. Ghiasvand y D. Parker, «Biochemistry of exercise-induced metabolic acidosis,» Am J Physiol Regul Integr Comp Physiol, vol. 287, 2004.

[3] F. M. Boffi, Fisiología del ejercicio en equinos, Buenos Aires: Intermédica, 2007.

[4] M. Dominguez, «Medbullets,» 21 07 2019. [En línea]. Available: https://step1.medbullets.com/respiratory/117022/carbon-dioxide-transport. [Último acceso: 2019 10 26].

[5] B. G. Klein, Fisiología veterinaria de Cuningham, Barcelona: Elsevier Saunders, 2014.

Fisiología cardiovascular, respiratoria y musculoesquelética durante la natación en caballos.

La natación aplicada al entrenamiento y rehabilitación de caballos data de la década de 1970, lo que significa que es un método empleado hace varios años que ha venido siendo estudiada en algunos aspectos, sin embargo, actualmente carece del estudio juicioso en caballos de disciplinas diferentes a la hípica. Los escenarios utilizados van desde el uso de piscina circulares y rectas hasta la natación en sitio (*tethered swimming*).

La natación o correr en el agua han sido utilizados durante mucho tiempo por entrenadores de caballos como métodos adicionales de entrenamiento. Los atletas humanos también han utilizado estos métodos, aunque principalmente para rehabilitación de lesiones.

Los caballos nadan en una piscina de forma circular o corren en una cinta sumergida o caminador. La frecuencia cardiaca (FC) tanto en los humanos como en los caballos es más baja cuando hacen ejercicio en el agua, en comparación con el ejercicio en tierra a una intensidad similar. Al parecer esta “bradicardia” obedece a la activación del reflejo trigémino-cardiaco (Jones et al., 2019). Puede ser un trabajo máximo o submáximo según la duración de las sesiones y la adaptación del animal al ejercicio. Han sido registradas concentraciones de lactato plasmático tan altas como 10 mmol/L y 200 latidos por minuto (lpm) (Boffi, 2007; Hobo et al., 1998). La frecuencia respiratoria ha sido reportada alrededor de 25 respiraciones por minuto (rpm) (Hobo et al., 1998; Jones et al., 2019).

El efecto de acondicionamiento en los caballos entrenados con natación puede atribuirse al aumento de la presión externa del agua sobre la cavidad torácica y la limitación de la capacidad respiratoria. Dada esta situación, la natación se ha considerado un entrenamiento ideal para el desarrollo de la musculatura respiratoria.

Tradicionalmente el uso principal de nadar y correr en agua es la rehabilitación de lesiones osteomusculares y para entrenar caballos jóvenes, ya que las propiedades de flotabilidad del agua disminuyen las fuerzas de concusión en los huesos y las articulaciones (Adair, 2011; Hodgson y Rose, 1994). A pesar de utilizarse como método para entrenar animales con lesiones, vale la pena mencionar que están restringidos aquellos animales con patologías del dorso y la pelvis, puesto que durante la natación el dorso es lordótico por causa del movimiento exagerado del miembro posterior (Adair, 2011).

La natación en caballos es bastante diferente de los humanos en relación con las respuestas fisiológicas al ejercicio. Los estudios en humanos han demostrado que el consumo de oxígeno (VO_2) más alto alcanzado durante la natación es aproximadamente el 90% del obtenido durante el ciclismo (Lucía et al., 2002). El factor principal que impide el uso de la natación de alta intensidad como en humanos para el caballo son las restricciones en su capacidad de respiración. Como resultado, la mayoría de los entrenadores permiten que los caballos naden a su propio ritmo (Davie et al., 2008).

Al tratarse de un animal respirador nasal obligatorio, el caballo modifica la forma como obtiene el aire ambiental evitando el ingreso de agua a las vías respiratorias durante la natación. Durante la natación el caballo experimenta episodios de apnea, colapso de los ollares con elevación del belfo superior además de colapso de las vías aéreas superiores. Aducción de los cartílagos aritenoides y colapso de las cuerdas vocales que resultan a su vez en cierre de la rima glotídica, y colapso en circunferencia de la nasofaringe. Se presume que la presencia de apnea durante la natación del caballo tiene por objetivo favorecer la flotabilidad, como manifestación del reflejo de buceo del mamífero. Sin embargo, a pesar de tratarse de un evento fisiológico desencadenado por la inmersión total en el agua, la presión arterial y el esfuerzo respiratorio durante la espiración llevan a algunos caballos a desarrollar hemorragia inducida por el ejercicio (Jones et al., 2019).

Estos cambios de presión pueden tener el potencial de crear un ambiente hipóxico que ejerza un estrés respiratorio en los caballos. Se sabe que la hipoxia es un estímulo clave para ciertas adaptaciones fisiológicas y puede ser un factor desencadenante de las adaptaciones al entrenamiento de natación (Davie et al., 2008). Al parecer la presión intratraqueal durante la espiración y la inspiración son similares, sin embargo, la presión de gases venosos y arteriales se modifican en función del consumo de oxígeno (Hobo et al., 1998), así, a medida que mejore la capacidad aerobia del animal bajo entrenamiento en piscina este comportamiento será más marcado.

Algunos estudios han intentado establecer la capacidad del entrenamiento en piscina de generar modificaciones estructurales cardiacas, sin embargo, en caballos de carreras a los cuales se les aplicaron protocolos de natación diseñados por entrenadores tradicionales no se encontró diferencia con el entrenamiento en piso (Davie et al., 2008). En un estudio realizado para determinar el efecto de la natación con baja carga de trabajo sobre el volumen sistólico se encontró que dicho parámetro pasó de 2.06 ml/kg en reposo a 1.5 ml/kg en natación. Esta respuesta parece estar relacionada con la disminución del retorno venoso, asociado a las alteraciones respiratorias durante la natación (Marr y Bowen, 2010). La inmersión en el agua produce disminución de la resistencia vascular sistémica, lo que favorece el trabajo cardiaco durante la natación (Adair, 2011).

Algunas de las características de la natación en los caballos son:

1. Se pierde la duración normal del ciclo respiratorio, la inspiración se acorta, pero se alarga la espiración. Este tipo de respiración es consecuencia de la presión ejercida por el agua sobre el tórax del caballo (Boffi, 2007; Hobo et al., 1998; Jones e Hiraga, 2006).

2. Se incrementa la presión arterial en comparación con el trabajo en piso (Boffi, 2007).
3. La sudoración es menor debido a la disminución en el requerimiento de termorregulación (Boffi, 2007).
4. Los músculos de los miembros torácicos permanecen activos, en comparación con el trabajo de trote y galope en piso (Boffi, 2007). No existe acoplamiento locomotor como sucede en el galope (Adair, 2011; Jones et al., 2019).

En cuanto a los efectos sobre la musculatura, se han reportado pocos estudios. Misumi et al. (1995), encontraron que al aplicar un protocolo de entrenamiento combinado (carrera y natación 300m/semana durante 2 meses y 500m/semana durante 3 meses) las fibras de contracción rápida altamente oxidativas (IIa) aumentaron de manera significativa comparado con el entrenamiento en carreras únicamente. Al parecer este tipo de entrenamiento favorece el metabolismo aerobio muscular.

Referencias bibliográficas

[1] Adair, H. S. Aquatic therapy for conditioning and treatment of tendon and ligament injuries, In Depth: integrative medicine (complementary and alternative medicine), 2011.

[2] Boffi, F. M. Fisiología del ejercicio en equinos, Buenos Aires: Intermédica, 2007.

[3] Davie, A.; Savage K.; Fennell, L. The Effect of Swimming Training on the Cardiac Dimensions in Thoroughbred Horses, Melbourne: Union Offset Printers, 2008.

[4] Hobo, S.; Yoshida, K.; Yoshihara, T. Characteristics of Respiratory Function during Swimming Exercise in Thoroughbreds. Journal of veterinary medical science. 60(6): 687-689, 1998.

[5] Hodgson, D. R.; Rose, R. J. Principles and practice of equine sports medicine: the athletic horse, Philadelphia: Saunders, 1994.

[6] Jones, J.; Hiraga, A. Metabolic, cardiovascular, and respiratory responses to swimming in horses, de Proceedings of a Workshop on exercise-induced pulmonary haemorrhage: state of current knowledge, Vancouver, 2006.

[7] Jones, S.; Franklin, S.; Martin, C.; Steel, C. Complete upper airway collapse and apnoea during tethered swimming horses. Equine veterinary journal, 2019.

[8] Lucía, A.; Hoyos, J.; Santalla, A.; Pérez, M.; Chicharro, J.L. Kinetics of $\dot{V}O_2$ in professional cyclists. Med. Sci. Sports Exerc. 34, 320–325, 2002.

[9] Marr C.; Bowen, M. Cardiology of the horse, Elsevier, 2010.

[10] Misumi, K.; Sakamoto, H.; Shimizu, R. Changes in skeletal muscle composition in response to swimming training for young horses. Journal of veterinary medical science. 57(5):959-961, 1995.

Capítulo 1. Respuestas hemáticas, metabólicas y cardiovasculares al ejercicio en un grupo de caballos colombianos de paso obesos.

El presente capítulo da respuesta al objetivo específico 1: *"Describir las respuestas hemáticas, metabólicas y cardiovasculares al ejercicio en piscina, en un grupo de CCC obesos".* Durante el desarrollo de la investigación se encontró que la información científica con respecto a las respuestas fisiológicas al ejercicio en caballos de paso es escasa, de modo que no se contaba con valores de referencia para comparar las respuestas fisiológicas provocadas por la natación. En consecuencia, se analizó información resultante del ejercicio en piso (pruebas de esfuerzo) de un grupo de caballos de paso colombianos, entre los cuales se encontraron incluidos los animales sometidos a natación. Este capítulo recopila las respuestas hematológicas, metabólicas, y cardiovasculares durante el ejercicio en tierra y en piscina.

Este capítulo está compuesto por dos artículos, uno ACEPTADO en la *Revista Mexicana de Ciencias Pecuarias* y otro PUBLICADO en la revista *Veterinary and Animal Science.*

Adicionalmente, información extraída parcialmente de los resultados fue plasmada en material para congresos: "*Resposta cardíaca de um cavalo crioulo colombiano ao exercício em piscina: relato de caso"* en el IX Simpósio Internacional do Cavalo Atleta 2019 de la Universidade Federal de Minas Gerais, Brasil; "*Limiar anaeróbio em cavalos da raça passo colombiano submetidos a prova de esforço a campo"* en el X Simpósio Internacional do Cavalo Atleta 2021 de la Universidade Federal de Minas Gerais; "*Hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian paso horses"* en el XVI Encuentro Nacional y IX Internacional de Investigadores de las Ciencias Pecuarias - ENICIP 2021 (Virtual).

UNIVERSIDAD DE ANTIOQUIA

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Hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian paso horses

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Guías para los autores:

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PARÁMETROS HEMATOLÓGICOS, BIOQUÍMICOS Y ENDOCRINOS EN RESPUESTA AGUDA AL EJERCICIO DE INTENSIDAD CRECIENTE EN CABALLOS DE PASO COLOMBIANO

HEMATOLOGICAL, BIOCHEMICAL, AND ENDOCRINE PARAMETERS IN ACUTE RESPONSE TO INCREASING-INTENSITY EXERCISE IN COLOMBIAN PASO HORSES

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Resumen:

El presente estudio tuvo como objetivo describir los parámetros hematológicos, bioquímicos y endocrinos en respuesta aguda al ejercicio de intensidad creciente en caballos de paso colombiano (CPCs). Se realizó una prueba de esfuerzo estandarizada en campo en 11 CPCs adultos no entrenados previamente y de ambos sexos. Las variables de interés fueron medidas antes y después de la prueba (i.e. hematocrito, proteínas plasmáticas totales, creatin kinasa, creatinina, nitrógeno ureico en sangre —BUN, aspartato aminotransferasa, gamma glutamil transpeptidasa, triglicéridos, colesterol, fosfatasa alcalina, cortisol, insulina, niveles de glicemia). Se encontró evidencia de la activación de la respuesta simpático-adrenérgica descrita para otras razas y disciplinas deportivas ecuestres (i.e. hemoconcentración, cambio negativo del volumen plasmático, incremento leve de la creatinina y del BUN). Además, hubo evidencia de movilización y utilización de fuentes de energía como glucosa y triglicéridos. Se concluye que el ejercicio de intensidad creciente realizado a partir de una prueba de esfuerzo en campo produjo un cambio negativo del volumen plasmático y la activación de la respuesta simpático-adrenérgica clásica en CPC.

Palabras clave: Entrenamiento, Esplenocontracción, Equino, Patología Clínica.

Abstract:

The present study aimed to describe the hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian Paso horses (CPHs). A standardized field exercise test was carried out on 11 untrained adult CPHs of both sexes. The variables of interest were measured before and after the test (i.e. hematocrit, total plasma proteins, creatine kinase, creatinine, blood urea nitrogen —BUN, aspartate aminotransferase, gamma glutamyl transpeptidase, triglycerides, cholesterol, alkaline phosphatase, cortisol, insulin, blood sugar levels). Evidence of sympathetic-adrenergic response activation, described for other breeds and equestrian sports disciplines (i.e. hemoconcentration, negative change in plasma volume, slight increase in creatinine and BUN) was found. In addition, evidence of mobilization and use of energy sources such as glucose and triglycerides was found. In conclusion, the increasing-intensity exercise carried out during a standardized field test produced a negative change in plasma volume and the activation of the classic sympathetic-adrenergic response in CPHs.

Key words: Clinical Pathology, Equine, Splenic contraction, Training.

Introduction

Standardized treadmill or field exercise tests have allowed to identify the horse's physiological responses and adaptations to exercise. Their characterization and interpretation would later become training indices[1,2].

Hematological and biochemical parameters are included within the group of variables of interest to be evaluated from such exercise tests. Nevertheless, what has been reported in this regard in horses may differ according to the intensity and duration of the exercise[3,4]. In addition, some findings are not considered as responses or physiological adaptations, but as exercise-induced disorders, including hemolysis and lymphopenia[5]. In Colombian Paso horses (CPHs), there are not enough reports to confirm the expected changes during exercise in animals of this breed.

Due to the increasing demand for professional accompaniment in the training of CPHs, it became important to describe the hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise for the breed.

Materials and methods

Ethical considerations

The procedures carried out on the animals of study were approved by the Comité de Ética para la Experimentación con Animales (CEEA) of the Universidad de Antioquia (Act #122, February 5, 2018).

Study location

The study was carried out in facilities located in a very humid lower montane forest life zone[6] (2,130 meters above sea level), with an environmental temperature between 12 and 18 °C, and a relative humidity of 96 %.

Animals

Eleven untrained adult CPHs were chosen at convenience. Nine non-pregnant females and two uncastrated males, with a mean of 6.6 ± 4.8 (2.5 - 16 y) of age, 371 ± 30 kg of weight and 7/9[7] of body condition were included. Animals were clinically healthy on physical examination, with a complete and updated health plan (vaccines and deworming) at the time of the measurements. Regarding the management conditions, the animals were under complete housing and fed on pangola grass hay (*Digitaria eriantha*; 2.5 kg/d on average), green forage (*Pennisetum purpureum*; 30 kg/d on average), commercial balanced feed (2 kg/d on average), mineral salt formulated for horses (100 g/d), and water *ad libitum*.

Field exercise test

A standardized field exercise test was carried out and was composed by four steps with increasing-intensity, also considering moments of rest and recovery. Heart rate (HR) was measured using a monitor reference Ambit 3 vertical (Suunto®, Finland). The protocol used [8] controlled the intensity of the exercise in each step (warm-up, 58 to 65 % of the maximum HR + moderate intensity, 65 to 75 % of the maximum HR + high intensity, 75 to 85% of the maximum HR + maximum intensity, ≥ 85 % of maximum HR).

Definition of the hematological, biochemical, and endocrine parameters

A venous blood sample was collected in a tube with EDTA for the measurement of hematocrit (HTC) and total plasma proteins (TPPs) during the moments of rest and at the end of each step of the exercise test. The percentage of change in plasma volume was determined by the concentration of albumin at the moment of rest and at the end of the exercise test [9].

In addition, samples were taken both in a tube with EDTA and in a dry one, at the moments of rest and of maximum intensity for the behavior of the complete blood count (CBC; i.e. total concentration of erythrocytes, leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, bands, platelets, hemoglobin, mean corpuscular hemoglobin concentration —MCHC, fibrinogen), and blood chemistry (i.e. creatine kinase —CK, creatinine, blood urea nitrogen —BUN, aspartate amino transferase —AST, gamma glutamyl transpeptidase —GGT, triglycerides, cholesterol, alkaline phosphatase—AP), hormones (i.e. cortisol, insulin) and blood glucose levels.

Statistical analyses

Descriptive results for non-parametric data were reported as (ME), (IQR), (SD), (CV) for each variable instead. The Wilcoxon signed-rank test or u-test for paired samples (non-parametric alternative to t-test) with a confidence level of 95 % was used to compare the mean range of two related or paired samples for each horse in the study and for each variable of interest. The statistical software Stata 16.0 (StataCorp, 2020, College Station, Texas, USA) was used for all analysis.

Results

Hematocrit, total plasma proteins, and plasma volume

The behavior of the HTC during the exercise test was consistent and homogeneous among the study animals, as shown by the low values of the SD for all the variables (Table 1). On the other hand, the TPPs showed slight changes during each step of the exercise test. Albumin was analyzed separately, and given the TPPs values, its values were relatively homogeneous.

The mean plasma volume change was -4.65 ± 8.16 L, although three of the animals registered a positive change.

Table 1: Descriptive results of the hematocrit, total plasma proteins, and albumin values in each step of the field exercise test performed on the Colombian Paso horses of study.

	Hematocrit (%)			Total plasma proteins (g/dL)			Albumin (g/dL)		
Moment/Step	ME (IQR)	SD	CV	ME (IQR)	SD	CV	ME (IQR)	SD	CV
Rest	37.9 (36.4 - 43.8)	4.52	0.11	6.52 (6.0 - 6.6)	0.42	0.07	3.42 (3.30 - 3.47)	0.18	0.05
Warm-up	42.0 (37.0 - 42.5)	4.62	0.11	6.62 (6.34 - 7.05)	0.41	0.06	3.51 (3.44 - 3.94)	0.28	0.08
Moderate intensity	48.0 (46.0 - 52.6)	5.09	0.11	6.96 (6.25 - 7.06)	0.46	0.07	3.63 (3.57 - 4.15)	0.36	0.10
High intensity	49.7 (47.0 - 53.9)	4.60	0.09	7.14 (6.32 - 7.25)	0.49	0.07	3.69 (3.60 - 3.99)	0.23	0.06
Maximum intensity	51.4 (50.3 - 54.8)	3.67	0.07	7.03 (6.49 - 7.13)	0.48	0.07	3.65 (3.63 - 4.00)	0.18	0.05
Recovery	41.5 (41.0 - 44.9)	2.81	0.07	6.77 (6.07 - 7.13)	0.49	0.07	3.60 (3.49 - 3.94)	0.24	0.07

ME = Median; IQR = Interquartile range; SD = Standard deviation; CV = Coefficient of variation. Reference value for hematocrit[10]= 32 - 47%; reference value for total plasma proteins[11] = 5.2 - 7.9 g/dL; reference value for albumin[11] = 2.6 - 3.7 g/dL.

Hematological parameters

In the hematological parameters before and after the exercise test, there were changes in fibrinogen (*P*= 0.004), the total concentration of leukocytes (*P*= 0.0475) and bands (*P*= 0.0002) (Table 2).

Table 2: Hematological parameters with significant statistical results, measured before and after the field exercise test performed on the Colombian Paso horses of study.

Hematological parameter	Moment	ME (IQR)	SD	CV	Ref. value [10]
Fibrinogen (mg/dl)	Before	200 (200 – 600)	214.9	0.58	100 - 500
	After	200 (200 – 500)	206.7	0.58	
Total concentration of leukocytes ($10^3/mm^3$)	Before	8.2 (7.1 – 10.5)	1.91	0.21	5.2 - 12.1
	After	9.3 (8 – 12.2)	2.26	0.23	
Total concentration of bands ($10^3/mm^3$)	Before	0.0 (0.0 – 0.08)	0.09	1.95	0 – 14
	After	0.0 (0.0 – 0.08)	0.06	2.5	

ME = Median; IQR = Interquartile range; SD = Standard deviation; CV = Coefficient of variation.

Biochemical parameters

The biochemical parameters were not statistically different (P>0.05) for the mentioned steps, with the exception of AP. However, some enzymes showed an increased activity in relation to the physiological concentration (i.e. CK, AST), as presented in Table 3.

Table 3: Biochemical parameters measured before and after the field exercise test performed on the Colombian Paso horses of study.

Biochemical parameter	Moment	ME (IQR)	SD	CV	Ref. value
Creatine kinase (U/L)	Before	250 (196 - 293)	56.01	0.224	90 - 270[12]
	After	279 (247 - 337)	79.11	0.263	
Creatinine (mg/dL)	Before	1.54 (1.44 - 1.62)	0.150	0.098	1.2 - 1.9[11]
	After	1.71 (1.62 - 1.99)	0.316	0.175	
Blood urea nitrogen (mg/dL)	Before	22.48 (20.37 - 24.45)	2.35	0.105	8 - 27[12]
	After	23.7 (22.54 - 26.7)	2.487	0.103	
Aspartate amino transferase (U/L)	Before	294 (263 - 341)	56.01	0.186	226 - 366[11]
	After	320 (270 - 356)	61.73	0.189	
Gamma glutamyl transpeptidase (U/L)	Before	15 (11.31 - 23.92)	5.49	0.338	4.3 - 13.4[11]
	After	18 (16 - 24.51)	6.55	0.322	

Triglycerides (mg/dL)	Before	26.12 (15.5 - 35.8)	23.35	0.707	11 - 52[12]
	After	54.3 (35.1 - 63.9)	14.26	0.280	
Cholesterol (mg/dL)	Before	114.1 (90.05 - 123.6)	23.10	0.213	51 - 109[12]
	After	102.25 (58.3 - 131.15)	40.60	0.393	
Alkaline phosphatase (U/L)	Before	343.21 (320.7 - 541) [a]	139.06	0.365	109 - 315[12]
	After	335.05 (321.9 - 488.18) [b]	131.90	0.335	

ME = Median; IQR = Interquartile range; SD = Standard deviation; CV = Coefficient of variation. [a, b] = Significant difference when each parameter was compared before and after the exercise test, according to the Wilcoxon signed-rank analysis ($P < 0.05$).

Endocrine parameters (hormones and glycemia)

Table 4 shows the hormonal profiles and blood glucose levels obtained during the study, which were not statistically different (P>0.05) for the mentioned steps.

Table 4: Behavior of cortisol, insulin, and glucose before and after the field exercise test performed on the Colombian Paso horses of study.

Endocrine parameter	Moment	ME (IQR)	SD	CV	Reference value
Cortisol (µg/dL)	Before	7.91 (2.21 - 14.39)	6.781	0.780	3.0 - 13[13]
	After	7.04 (5.19 - 9.61)	7.318	0.773	
Insulin (µIU/mL)	Before	40.98 (19.16 - 55.96)	50.84	1.067	4.52 -33.53[14]

	After	26.47 (22.92 - 54.56)	18.60	0.535	
Glucose (mg/dL)	Before	101 (85 - 153)	35.69	0.313	71 - 130[14]
	After	134 (101 - 162)	37.89	0.286	

ME = Median; IQR = Interquartile range; SD = Standard deviation; CV = Coefficient of variation. [a, b] = Significant difference when each parameter was compared before and after the exercise test, according to the Wilcoxon signed-rank analysis (*P* > 0.05).

Discussion

Acute responses to exercise, both hematological, biochemical, and endocrine, have been scarcely reported in CPHs. Therefore, the acute physiological changes that occur during exercise in this breed are currently not recognized. This situation represents a disadvantage for the professionals who participate in the sports conditioning processes, since it compels them to work from references that do not correspond to the context of the CPHs. On the other hand, the absence of such information enables the erroneous interpretation of findings in blood tests, although some of the hematological and biochemical variations provide a significant perspective of the pathological conditions that occur from intense exercise[15]. Also, it is important to consider that the hematological, biochemical, and endocrine responses by themselves do not describe the athletic capacity of the horse; in fact, parameters such as HTC have not been found to be correlated with metabolic indicators por example the anaerobic threshold[16].

It is pertinent to clarify that the reference values considered for the present study were contrasted with previous studies carried out in animals of the same breed. The above corresponds to the hemoleukogram values[10], cortisol and insulin concentration[12-14]. The other variables were contrasted with literature[11,12]. The HTC was measured and not calculated, despite using automated equipment.

Exercise is an event of physiological stress, for this reason it is expected the HTC to increase, mainly in moderate, high, and maximum intensity, as occurred in the animals of the present study, exceeding the reference values for rest during the mentioned steps. The behavior observed for HTC is explained by splenic contraction and loss of water during exercise, reflecting hemoconcentration. It takes 30 to 60 seconds for splenic erythrocyte release to occur in the presence of increased circulating epinephrine. During the recovery, the sequestration of erythrocytes and leukocytes by the spleen takes approximately five minutes, although their full reserve can take up to 30 minutes[15]. Thus, the return of HTC to its value at rest or during warm-up is explained by this phenomenon, in addition to the blood volume recovery.

In view of the fact that HTC is highly affected by the adrenergic response during exercise (to establish polycythemia due to dehydration), it is recommended to include the total concentration of erythrocytes in the analysis and compare it with the HTC and the concentration of TPPs. In this group of horses, both events were found (hemoconcentration due to splenic release and decrease in plasma volume), as a consequence of dehydration.

The TPPs and albumin showed an increasing behavior during exercise and were found to be augmented (within the reference range) due to the aforementioned loss of water. The concentration of plasma proteins at rest and during exercise, is the result of the interaction of numerous factors, such as the degree of filtration between the intra and extravascular spaces, metabolic demands, neuroendocrine control, nutritional status, and water balance(17).

The plasma volume change found in the present study may be related to the movement of fluids between the different compartments, given the increasing of the hydrostatic pressure generated by the rise in arterial and venous pressure during exercise. In addition, it could be related to the secretion of natriuretic peptide, as the intensity of exercise increases(15).

In humans, it has been reported that plasma volume can decrease due to hemoconcentration or even increase due to hemodilution, depending on the type of exercise performed. The decrease in plasma volume is greater during high intensity exercise(18,19), and the magnitude of sweating and hydration during exercise can also determine its decrease(20). In the present study, a reduction in plasma volume was observed at the end of the exercise test, when horses showed profuse sweating. This corroborates that, as in humans, high intensity exercise and sweating reduce this parameter.

A previous study reported an increase of 5.12 % in plasma volume in endurance horses that competed in an 80 km race, hydrated with 30 L of a solution with sodium chloride and potassium chloride, while, in hydrated horses (with 10 L of the same solution), a decrease in plasma volume of -2.34 % was observed[21]. In this study, the change in plasma volume was -4.65 %, which was expected, since the horses did not hydrate until the final of the exercise test and the sampling were completed. Starling forces explain these findings based on changes in hydrostatic and oncotic pressures in the vascular and interstitial compartments. During physical activity, a redistribution of cardiac output occurs. Blood flow to the active musculoskeletal system and to the skin tissue increases, and the rise in capillary hydrostatic pressure favors the passage of water and even proteins to the interstitial compartment. These events explain how fluid movement affects plasma volume during exercise[19].

The leukogram obtained from the animals in the present study was significantly different when the values obtained were compared in the rest and recovery steps. Leukocytes show transient alterations in response to increased sympathetic tone. When stored with red blood cells in the spleen, splenic contraction can lead to an increase in the count by approximately 30%[15]. However, its increase is not an indicator of physical condition, rather it is the neutrophil:lymphocyte ratio (10:1) with a shift to the left, is a sign of exhaustion, stress, or overtraining. In the horses of the present study, bands were found in three of the animals, without changes in the neutrophil:lymphocyte ratio. It was not considered a pathological finding, since the values were within the reference range, and it was not accompanied by other changes in the leukogram or at the clinical examination. In addition, values around 50 % of the total neutrophils are sequestered in the capillary spleen beds and are known as marginal pool or splenic reserve. Marginalized neutrophils can be mobilized under certain conditions, including exercise, stress, transport, and exogenous corticosteroids or catecholamine administration, causing variations in the leukogram[22].

Within the biochemical values analyzed herein, the fibrinogen in some animals was found to be remarkable. It should be taken into account that fibrinogen is an acute phase protein, considered as a nonspecific indicator of inflammation[23]. It is presumed that some of the animals in the present study experienced some process related to ongoing inflammation that was not reflected in the clinical examination, nor could it be related to leukocyte values. It is understood that the increase in fibrinogen is related to inflammatory processes of infectious or non-infectious origin, so the albumin:globulin ratio may clarify the origin of such increase. In addition, fibrinogen synthesized in the liver in response to an inflammatory process may remain increased —even when the lesions resolved several days ago, registering a peak between 5 and 7 days after the lesion. Therefore, fibrinogen is an indicator of inflammation with absent hemoconcentration.

Blood biochemistry analyzes of the animals in the present study demonstrated that the effort printed on the test is sufficient to increase muscle biochemical activity. It is advisable to add a post-recovery measurement to check if the reestablishment of blood volume alters the concentration of related analytes or if these remain elevated as indicators of muscle injury. The post-test eligible time to detect underlying damage should be anticipated based on the analyte. In racehorses, it is known that 3 days after a competition, some hematic and biochemical values have not yet returned to their reference range[24].

Creatinine tends to increase during exercise, due to an augmented use of phosphocreatine and gluconeogenesis, and a decreased glomerular filtration rate[15], being a confident indicator of muscle metabolism and kidney function. The increased use of phosphocreatine evidences the high and maximum intensity work that the study horses experienced.

The BUN did not show a specific trend. This was expected from this parameter since BUN is the result of the urea cycle and the metabolism of nitrogenous products obtained from the diet. Furthermore, it can be reabsorbed in the proximal convoluted tubule in about 30 %, therefore, it is not a good indicator of functionality nor is it a good index of metabolism to be observed after exercise.

The CK is the enzyme in charge of hydrolyzing the reaction that produces ADP and phosphocreatine from ATP. Its increase is mainly associated with a rise in cell permeability due to acidosis or an increase in the use of ATP by the musculoskeletal system. The peak of its production occurs 4 to 12 hours after the event that triggers it. The animals in the present study registered an increase in the blood concentration of this enzyme; however, no animal registered an increase related to injury. Nevertheless, no samples were taken during the described release peak, which limits the inference from this enzyme.

Despite being present in several organs, AST is used as a marker of cellular injury in the liver or the musculoskeletal system. Its magnification must be 5 to 100 times greater than the reference value to be useful from the clinical point of view[(15)]. This strengthens the premise that the field exercise test applied to the animals of the present study does not constitute a harmful activity for the musculoskeletal system in healthy horses without previous training.

The biochemical analysis also allowed the recognition of the energy sources that the horses used for this kind of effort. An increased mobilization of glucose and triglycerides was observed. During exercise, glycolysis and lipolysis are activated to obtain energy for muscle contraction[(15, 25)], especially under aerobic conditions, as occurred in the warm-up and other steps of the exercise test used herein.

It is advisable to include bilirubin quantification to verify the presence of erythrocyte rupture due to the fragility of the membrane that can occur because of changes in blood pH derived from exercise. Hence the importance of accompanying this analysis with the measurement of MCHC. In the present study, bilirubin was not measured, however, MCHC remained within the reference range.

The hormonal activity during exercise is related to the body's energy requirements. The activation of specific endocrine responses is highly dependent on the intensity and duration of exercise, which attempt to preserve the life of the animal through the use of multiple metabolic mechanisms to provide additional energy for muscle contraction[26].

Glycogenolysis and gluconeogenesis are activated during exercise, in part, by the release of cortisol, growth hormone, and catecholamines[27], while insulin secretion is decreased by the release of catecholamines during exercise[15]. In contrast, other authors found that exercise does not influence insulin secretion[28]. According to the results of the present study, this hormone was found to be elevated before and after the field exercise test. Therefore, metabolic disorders should not be ruled out in the study animals, which were "overweight" at the time of the test, according to the body condition assessment (7/9 on average).

The physical and psychogenic stimuli associated with exercise induce the synthesis and secretion of adrenocorticotropic hormone (ACTH), β-endorphins, and cortisol. In addition, vasopressin — released during physical activity, enhances ACTH secretion. Cortisol measurement should be based on the circadian rhythm and breed, as reported for CPHs[13]. In horses without adequate athleticism, elevated serum cortisol levels affect leukocyte function[15]. However, this condition will remain as long as the exercise is strenuous. Contrary to expectations, in the case of the CPHs of study, the serum cortisol concentration remained within the reference intervals, even after the exercise test, although with a plasma concentration sufficient to explain the hyperglycemic and hyperlipemic observed effects.

Future studies on CPHs´ sports medicine should propose the identification of physiological changes triggered by physical effort, and thus determine the starting point for the detection of pathologies or as a point of comparison after the application of a prescribed training.

Conclusions

The increasing-intensity exercise performed by the CPHs in the present study produced water loss, as evidenced by the change in plasma volume, with the consequent hemoconcentration, and the slight increase in both creatinine and BUN. The significant differences observed in the leukogram and fibrinogen were apparently produced by individual factors of some animals. Therefore, it cannot be concluded whether the exercise test actually produces physiological responses in this regard. In addition, evidence of anticipated stress response was found from the cortisol value, in the absence of muscle injury. The CPHs considered in the study were suspected of equine metabolic syndrome, although they were not diagnosed for it. These factors must be taken into account when interpreting acute responses and adaptations derived from physical training in horses of this breed.

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Conflict of interests

The authors declare that they have no conflict of interest.

Literature cited:

1. Fraipont A, Van Erck E, Ramery E, Fortier G, Lekeux P, Art T. Assessing fitness in endurance horses. Can Vet J 2012; 53(3):311-314.

2. De Mare L, Boshuizen B, Plancke L, De Meeus C, De Bruijn M, Delesall C. Standardized exercise tests in horses: current situation and future perspectives. Vlaams Diergeneeskd Tijdschr 2017; 86(2):63-72.

3. Lekeux P, Art T, Linden A, Desmecht D, Amory H. Heart rate, hematological and serum biochemical responses to show jumping. In: Persson SGB *et al*. ICEEP Publications: Equine exercise physiology 3. 1991; pp. 385-390.

4. Zobba R, Ardu M, Niccolini S, Cubeddu F, Dimauro C, Bonelli P, *et al*. Physical, hematological, and biochemical responses to acute intense exercise in polo horses. J Equine Vet Sci 2011; 31(9):542-548.

5. Masini A, Tedeschi D, Baragli P, Sighieri PC, Lubas G. Exercise-induced intravascular haemolysis in standardbred horses. Comp Clin Path 2003; 12:45-48.

6. Holdridge LR, Grenke WC, Hatheway WH, Liang T, Tosi JA. Forest environments in tropical life zones: a pilot study, Pergamon Press, 1971.

7. Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983; 15(4):371-372.

8. Arias MP, Maya JS, Arango L. Efectos de dos protocolos de entrenamiento sobre el lactato sanguíneo en caballos de paso fino. Rev Med Vet Zoot 2019; 66(3):219-230.

9. Van Beaumont W, Strand JC, Petrofsky JS, Hipskind SG, Greenleaf JE. Changes in total plasma content of electrolytes and proteins with maximal exercise. J Appl Physiol 1973; 34(1):102-106.

10. Castillo CA, Tobón M, Cano CA, Mira J, Suárez AP, Vásquez EM. Valores hematológicos en caballos criollos colombianos del valle de Aburrá. In: Corporación Universitaria Lasallista editors. Perspectivas y avances de investigación de la serie Lasallista investigación y ciencia. 1st ed. Caldas, Antioquia, CO: Delasalle; 2010:245-262.

11. Kaneko J, Harvey J, Bruss M. Clinical biochemistry of domestic animals. 6th ed. David, USA: Academic Press; 2008.

12. Southwood L. Practical Guide to Equine Colic. 1st ed. Iowa; USA: Wiley-Blackwell; 2013.

13. Zuluaga AM, Martínez JR. Serum cortisol concentration in the Colombian creole horse. Rev Colomb Cienc Pecu 2017; 30(3):231-238.

14. Rosas M. Diagnóstico de síndrome metabólico equino y su asociación con signos clínicos de laminitis en el caballo criollo colombiano en la sabana de bogotá [master thesis]. Bogotá, DC: Universidad de la Salle; 2017.

15. Boffi F. Fisiología del ejercicio en equinos, 1st ed. Buenos Aires, Argentina: Intermédica; 2007.

16. Valette JP, Barrey E, Wolter R. Multivariate analysis of exercise parameters measured during an incremental treadmill test. In: Persson SGB *et al*. ICEEP Publications: Equine exercise physiology 3. 1991; pp. 337-342.

17. Mesa-Rojas MC. Análisis del comportamiento de los parámetros hematológicos en caballos que compiten en carreras de enduro a 2640 M.S.N.M. [undergraduate thesis]. Bogotá, DC: Universidad de La Salle; 2016.

18. Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 2000; 32(2):332-348.

19. Sanchis-Gomar F, Lippi G. Physical activity - an important preanalytical variable. Biochem Med 2014; 24(1):68-79.

20. Mora-Rodríguez R, Fernández VE, Hamouti N, Ortega JF. Skeletal muscle water and electrolytes following prolonged dehydrating exercise: Fluid losses in exercising skeletal muscle. Scand J Med Sci Sports 2014; 25(3):274-282.

21. Sampieri F, Schott HC, Hinchcliff KW, Gero RJ, Cunilleras JE. Effects of oral electrolyte supplementation on endurance horses competing in 80 km rides. Equine Vet J 2006; 38(36):19-26.

22. McKenzie EV. Hematology and serum biochemistry of the equine athlete. In: Kenneth H, Andris K, Raymond G editors. Equine Sports Medicine and Surgery. 2nd ed. USA: Elsevier Health Science; 2014; pp. 921-929.

23. Polla BS, Cossarizza A. Stress proteins in inflammation. EXS 1996; 77:375-391.

24. Bos A, Compagnie E, Lindner A. Effect of racing on blood variables in Standardbred horses. Vet Clin Pathol 2018; 47(34):625-628.

25. Bayly WM, Allen JR, Gollnick PD. Equine exercise physiology I: energy production and substrate utilization. Equine Vet Sci 1984; 4(3):114-118.

26. Hinchcliff KW, Geor RJ, Kaneps AJ. Equine exercise physiology: the science of exercise in the athletic horse, Elsevier Saunders, 2008.

27. López-Chicharro JL, Fernández-Vaquero A. Fisiología del ejercicio, Ed. Panamericana, 3ª edición, 2006.

28. Noble GK, Sillence MN. Diurnal rhythm and effects of feeding, exercise and recombinant equine growth hormone on serum insulin concentrations in the horse. Equine Vet J 2013; 45(6):745-750.

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Blood lactate concentrations and heart rates of Colombian Paso Horses during a field exercise test

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ABSTRACT

Information on performance indices in paso horses is scarce. Field exercise tests are necessary to recreate the exertion that occurs during training and competition.

To describe blood lactate concentrations and heart rate of untrained Colombian Paso Horses (CPHs) in response to a field exercise test.

A 30-minutes duration standardized field exercise test was carried out on 11 untrained adult CPHs of both sexes. The variables of interest (BL concentration and heart rate —HR) were measured before, during each step of the test, and at recovery. The HR and BL were used to calculate the HR at which a BL of 4 mmol/L or anaerobic threshold (HRL_4) was reached.

The HR during the field exercise test increased according to the protocol used. The BL concentration during the test was variable and, despite having been increasing like the HR, the distribution of the values in each step of the test was remarkably dispersed. The mean BL clearance percentage was 56.3 ± 16, similar in most of the animals. The anaerobic threshold was reached at a notably different HR between individuals (132 and 251 bpm).

The field exercise test protocol used herein is useful to assess heart rate and BL changes in acute response to exercise in CPHs. It would be useful to evaluate training kinetics with other parameters including blood count and muscle enzymes.

Keywords: equine, exercise, heart rate, lactate

1. Introduction

The knowledge of the specific physiological effects of the effort the horse experiences in each sport discipline allows improving the conditioning and training processes. In addition, its biological characterization is a key determinant of performance indices, used to identify the appropriate physical state (Allen et al., 2016). The physical training of horses induces physiological adaptations necessary to compete and withstand the intensity of the different tests, with a minimum risk of injury and ensuring appropriate behavioral and psychogenic indicators (Kupczyńsk et al., 2018).

Standardized treadmill tests have been widely used for this purpose, allowing the animal to become fatigued by increasing the speed and slope of the treadmill, thus determining the maximum effort and metabolic expenditure required (Allen et al., 2016).

In paso horses, the use of the treadmill is limited, as in other equestrian disciplines (Allen et al., 2016; Fraipont et al., 2012), since it is not possible to replicate the gait pattern (Novoa et al., 2021) of these animals without the rider. The use of the treadmill in CPHs is not possible because the gait performance occurs when the bit interacts with the horse's mouth, in addition, the rider is usually overweight increasing the intensity of the exercise. Therefore, it is necessary to evaluate paso horses through field-based tests, allowing to recreate the exertion that occurs during training and competition, generating performance indexes for the sporting discipline.

A performance test is essential in the development of a training program for the sport horse because it allows to determine the athletic state of the animal based on the physiological variables related to cardiac and metabolic activity, in addition to facilitating the evaluation of the effects of exercise on the acid-base status. This test facilitates the characterization of the athlete and allows training strategies to be focused on reducing weaknesses and strengthening athletic abilities. Some researchers have described methods that allow evaluating the horse's progress throughout training, such as cardiac stress tests, and hematological and biochemical profiling (Piccione et al., 2007). The evaluation of the impact of exercise through blood parameters can provide practical information on the effectiveness of a training program (Kupczyński et al., 2018) and allows to determine if there are cardiovascular, hematological, and metabolic alterations induced by it. However, the changes derived from exercise in each discipline and breed are different and must be characterized specifically.

It is not clear whether the CPH reaches the anaerobic threshold when the HR is close to 200 bpm as has been described in racehorses (Hodgson et al., 2014). Nor is it known if the BL increases proportionally with the heart rate.

Therefore, the anaerobic threshold for CPH has not been reported. Traditionally, the anaerobic threshold has been calculated using the speed at which the horse reaches the BL of 4 mmol / L, however, in the case of CPHs this is not possible due to the differences in its gait performance. For these horses it is not useful to measure V_4 as it is done in other equestrian disciplines, because speed (distance covered per unit of time) does not increase as a result of improved performance. When a CPH improves its performance, it increases the number of strokes or steps per minute independent of the distance traveled. For this reason, to establish the moment in which the animal exercises under anaerobic metabolism, the heart rate should be used instead of the speed.

Hematological, acid-base, and metabolic changes that occur as a result of a maximum-intensity exercise have been characterized in some equestrian sports, through standardized field tests (Fraipont et al., 2012; Munsters et al., 2014; Fortier et al., 2015), while for the Colombian paso horse (CPH) this information is not available so far. In this sense, this study aimed to describe blood lactate concentrations and heart rates of untrained Colombian Paso Horses in response to a field exercise test.

2. Materials and methods

2.1. Ethical declarations

The procedures carried out on the animals of study were approved by the Comité de Ética para la Experimentación con Animales (CEEA) of Universidad de Antioquia (Act #122, February 5, 2018).

2.2. Study location

The facilities where the study was carried out correspond to A "Very Humid Lower Montane Forest" life zone (Holdridge et al., 1971), located at 2,130 meters above sea level, with an environmental temperature between 12 and 18 °C, and a relative humidity of 96 %.

2.3. Animals

Eleven untrained (at least six months) adult CPHs were chosen at convenience. Nine non-pregnant females and two uncastrated males, five horses paso fino gait, one horse trot gait and five horses trocha gait, with a mean age of 6.6 ± 4.8 (2.5 - 16 y), a mean weight of 371 ± 30 kg, and a mean body condition score of 7/9(8) were included in the study. Animals were clinically healthy on physical examination (data not showed), with a complete and updated health plan (vaccines and deworming) at the time of the measurements. Regarding the management conditions, the animals were under complete housing and fed on pangola grass hay (*Digitaria eriantha*; 2.5 kg/d on average), green forage (*Pennisetum purpureum*; 30 kg/d on average), commercial balanced feed (2 kg/d on average), mineral salt formulated for horses (100 g/d), and water *ad libitum*.

2.4. Field exercise test

A 30-minutes duration standardized field exercise test, composed by four steps with increasing-intensity, including rest periods and a final recovery was carried out. The heart rate (HR) was measured using a Ambit 3 sensor (Suunto®, Finland). The heart rate monitor (HRM) was validated with another HRM and an ECG equipment (data non-published). The protocol used consider controlling the intensity of the exercise at each step (warm-up, 58 to 65% of the maximum HR + moderate intensity, 65 to 75% of the maximum HR + high intensity, 75 to 85% of the maximum HR + maximum intensity, ≥ 85% of maximum HR) (Arias et al., 2019) (Table 1). The theoretical maximum HR reported for the breed is 221 ± 17 beats per minute (bpm) (Arias et al., 2006).

The track used had dimensions of 35 m in length and 20 m in width, the surface was covered by dry silt, like the tracks used in competitions. Under these conditions, 5 continuous minutes of exercise was guaranteed in each stage of the f-SET.

2.5. Blood lactate concentration and clearance curve

A venous blood sample was obtained from the jugular vein and collected in a tube with EDTA, using a vacuum system. Samples were collected at rest, during each step, and 10 minutes after the end of the test (recovery). A total of 10 µl were used to measure the BL using the Nova

Plus® portable device (Nova biomedical, USA). The HR and BL were used to calculate the HR at which a BL of 4 mmol/L or anaerobic threshold (HRL_4) was reached. The BL clearance was calculated using the BL concentration at the end of the maximum intensity step (BL_{max}) and the concentration at the recovery (BL_{recov}), as follows: $[(BL_{max} - BL_{recov})/BL_{max}]$.

2.6. Statistical analyses

Descriptive results were reported for all variables. In addition, the Wilcoxon signed-rank test or *u*-test for paired samples (non-parametric alternative to t-test) with a confidence level of 95% was used to compare the mean range of two related or paired samples for each horse in the study and for each variable of interest. None of the data groups had normal behavior. The statistical software Stata 16.0 (StataCorp, 2020, College Station, Texas, USA) was used for all analysis. The HRL_4 was calculated using the equation produced by the scatter plot and the exponential trend generated in Microsoft Excel® as previously reported by the literature (Fraipont et al., 2012). The R^2 of the equations obtained ranged between 0.82 and 0.97.

3. Results

3.1. Heart rate

The HR increased according to the protocol used for the field exercise test (Table 1). The HR during recovery was similar to that during warm-up (Figure 1).

3.2. Blood lactate concentration

The BL concentration during the field exercise test was variable. Despite having been increasing like the HR, the distribution of the values in each step of the test was remarkably dispersed. Elevated BL values were found in some of the individuals during the moderate, high, and maximum intensity steps (Figure 1), which affected the median value of each step, as can be seen in the interquartile range, standard deviation, and coefficient of variation reported herein (Table 2). Neither the HR nor the BL concentration were significantly different when the medians of the test steps were compared. In addition, it was determined that the mean BL clearance percentage was 56.3 ± 16.

3.3. Anaerobic threshold and blood lactate clearance

The anaerobic threshold was reached at a notably different HR between individuals (132 and 251 bpm). In addition, the BL clearance produced during the field exercise test was similar in most of the animals (Table 3).

4. Discussion

CPH is the most popular breed in Colombia and has been scarcely studied as an athlete. However, it has recently been included within the group of athlete horses since when it performs in its gait, it experiences a physical effort of moderate to maximum intensity (Arias et al., 2006) and receives arduous training to execute it. Some physiological characteristics related to the physical working capacity (PWC) of this type of horse have been described, such as its muscular composition (Enríquez et al., 2015) and the average and maximum HR (Arias et al., 2006). There are no other findings related to PWC for the breed, although theoretically and in accordance with what has been described in other equestrian disciplines, it is also determined by the size of the heart (Young et al., 2010) and the horse´s willingness to work (McBride and Mills, 2012).

Research on CPHs have reported HRs according to gait and have defined a theoretical maximum HR of 221 ± 17 bpm (Arias et al., 2006). Nevertheless, the information remains scarce and does not allow the training protocols to be designed on a technical and specific basis. Furthermore, little is known about the physiological limits that are reached with the performance of these sports activities. It is relevant to develop research around the characterization of these animals´ athletic activity to improve their performance and well-being conditions.

Although HR_4 is not as widely used a parameter as VL4, it is valuable when the displacement speed does not reflect an improvement in sports performance, especially in horses that perform activities as particular as CPH. During competitions, the judges do not evaluate how long a participation last. It has been argued that HR_4 is an interesting value in that it can be interpreted at different ages of the horse regardless of which stage of training it is in (Couroucé et al., 2002).

The present study has confirmed that the field exercise test is a useful approach to evaluate the cardiac and metabolic response in CPHs using few and inexpensive equipment, as has been reported for enduro horses under field and treadmill-based protocols (Fraipont et al., 2012). The study reported that the aerobic threshold (VL_2 and HRL_2) and anaerobic threshold

(VL_4 and HRL_4) in Enduro horses were different under treadmill and field-based protocols, despite having been studied in the same group of animals. Such results indicate that the presence of the rider is decisive in the intensity of the exercise. In the CPHs of the present study, the effect of the rider on exercise intensity was not explored, which should be an aspect to investigate in depth (Allen et al., 2016).

As previously reported, the maximum HR of each horse in the present study was different. This situation results from individual peculiarities in cardiac capacity and size and mental willingness to work (central fatigue) (McBride and Mills, 2012). It should be noted that not all individuals reached the theoretical maximum HR for the paso horse (221 ± 17 bpm); therefore, it is important to determine the individual maximum HR of each animal, and thus design the training in consideration of working volumes that are equivalent between individuals, using methods such as relative HR, percentage of HR reserve (%HRR), or percentage of oxygen consumption reserve (%VO_2R) (Mann et al., 2013). In addition, it is indicated to maintain the increasing intensity of exercise to adequately visualize the physiological changes that are happening, with the sequential activation of the response mechanisms to physical effort. The above highlights the importance of validating field exercise tests that are reproducible and specific for the type of physical effort performed by a certain group of competing horses. Untrained horses were used because it was not known for the breed what were the physiological responses to exercise at different intensities, therefore, there was no baseline information to evaluate the evolution in the application of any training protocol. On the other hand, CPHs are usually trained under different conditions and intensities according to the expertise of the riders even in the same stud farm. Therefore, trained animals have previously non-controlled physiological adaptations which produce bias to the study.

Changes occurring under controlled conditions are the subject of other research work.

After 10 minutes of recovery, the HR did not seem to agree with the HR obtained at rest, although it was similar to the one obtained during warm-up. This finding is explained by the phenomenon of cardiac recovery, which occurs in two phases rapid recovery (adrenal sympathetic response) and slow recovery, that in conjunction with the normalization of blood volume, splenic contraction, and thermoregulation can take a few hours to return to the frequency and rhythm of rest of the animal (Lindner et al., 2020). This is how the HR recovery is usually rapid in the first minute after exercise (Evans, 2007), and then slowly decreases over several hours (depending on the intensity of the exercise) until reaching the resting values (Lindner et al., 2020). In addition, the HR recovery time at rest values has been reported to be greater in untrained horses compared to trained ones (Hodgson et al., 2014), as could be registered in the CPHs of the present study.

Handling of horses after exercise also determines HR recovery time. Horses that walk have been reported to have a faster recovery at rest than those that remain inactive (Lindner et al., 2020). In the present study, the horses walked with the rider for the entire recovery time.

In the case of CPHs, adaptation to different environments is essential, since, when evaluating its HR, it is not altered by external factors such as noise or the presence of other animals. According to the authors' experience, the temperament of these horses and their response to external stimuli influence HR variations that are not characteristic of the physical effort carried out *per se*.

As the field exercise test increases, the HR increases linearly with respect to the displacement speed in horses other than paso ones, due to the increase in the release of catecholamines associated with exercise (Hodgson et al., 2014; Nagy et al., 2014). In contrast, in CPHs the HR increases in relation to exercise time and with speed of walking-related effort, measured in the number of strokes per minute.

According to the authors' experience, HR can also increase depending on the surface on which the horse exercises, since the sound of its horseshoes produces a state of excitement. The present study was carried out in a closed space, without an audience and without the use of the resonance track to avoid that effect.

The stable state was not identified or interpreted during the present study, since this parameter must be reached by the animal spontaneously. Nevertheless, the exercise intensity was controlled through HR monitoring during the test.

Lactate production was markedly different between individuals and between test steps. The study group consisted of untrained animals, of which differences in aerobic capacity for athletic performance are expected and, therefore, differences in the use of anaerobic metabolism during exercise are expected as well. The lactate values recorded in the high and maximum intensity steps agree with those reported in the literature for horses under field tests (Capacho and Arias, 2019) and exceeded the anaerobic threshold (4 mmol/L) as recommended in a maximal intensity test, which means that metabolic behavior is related to the intensity of exercise in this type of animal.

The BL measurement must be preceded by an adequate pre-analytical phase. The use of venous or arterial blood is indistinct, but the type of container, additive, and storage time and temperature of the sample is decisive. Red blood cells obtain energy (as ATP) only through anaerobic glycolysis due to the absence of mitochondria (Boffi, 2007), and when using plasma glucose, lactic acid is produced, among other residues. For this reason, it is recommended to perform the measurement immediately after taking the sample in a vacuum tube with EDTA, using a portable meter (Allen et al., 2016; Henderson, 2013), as done in the present study. Delays in testing can result in lowered glucose values and falsely elevated BL values.

The BL concentration in a horse at rest must be below 1 mmol/L (Allen et al., 2016; Henderson, 2013). In fact, in CPHs the resting BL concentration has been reported before and after applying two training protocols, with 0.92 mmol/L as a maximum value (Arias et al., 2019). Small increases in this concentration occur as the intensity of the exercise increases, and at the moment in which the production exceeds the removal, the concentration of BL increases exponentially (Allen et al., 2016; Nieto and Espinosa, 2009).

The rate at which lactic acid accumulates depends on animal inherent factors, including the cardiac delivery rate of oxygen to the working muscle (DO_2), the ability of the muscle cell to extract oxygen (VO_2), and the rate of lactate metabolism in the muscle cell during exercise. These factors are limited by the physiological characteristics of the horse as an individual —and also by age, but can be improved with training (Nieto and Espinosa, 2009). These physiological differences between individuals explain why both BL concentration and clearance were different in the study animals. The study group included young and adult animals, of which it is known that some muscle fibers can change the type of metabolism depending on the training received (Enríquez et al., 2015), which could explain the results obtained herein. Hence the importance of individually performing the field exercise test and avoiding transferring results from one animal to another, despite the fact that they are under similar handling conditions. In addition, it is advisable to measure directly and not calculate variables such as HR, BL, and VO_2 (Allen et al., 2016).

Through incremental exercise tests, the HR and the rate at which lactate accumulation begins above 4 mmol/L (OBLA) is determined. At this point, it is considered that anaerobic metabolism predominates in energy generation (Nieto and Espinosa, 2009). In CPHs, the incremental test allows knowing the anaerobic threshold, but not as a function of the speed of movement but rather of the intensity of the exercise. For this reason, the performance indicator used in the present study was HRL_4. This occurs because the speed of movement is not related to the athletic performance in paso horses, and therefore, it is not directly related to the intensity of the exercise.

The reaching of the anaerobic threshold is indicative of an imbalance between anaerobic cellular metabolism and the reuse of lactate. Most of the study animals reached the anaerobic threshold below 200 bpm, and some of them even reached it when they were exercised at low intensity. This means that their ability to exercise without fatigue was low for about 30 minutes (test duration). This information is relevant for this type of animal, since they are usually used for horseback riding in which long distances are covered and periods of more than 30 minutes are performed in consideration of the gait (Palacio et al., 2013).

The speed to reach the anaerobic threshold in some of the CPHs in the study can be explained by the high proportion of type II muscle fibers, as has been previously reported for paso horses (Enríquez et al., 2015). Despite rapidly reaching the anaerobic threshold, most of the study animals showed lactate clearance close to 60%. This can be explained by variations between the proportion of muscle fibers IIA and IIX of each horse and by the oxidative capacity of type I and IIA fibers, capable of reusing lactate as an energy source during recovery through gluconeogenesis, and its consequent removal of blood circulation (Enríquez et al., 2015). The interpretation of lactate concentration must be made according to its metabolism, given its synthesis and removal. Since lactate is a carbohydrate, its blood concentration is dynamic and it can be reused through hepatic and muscular gluconeogenesis (Henderson, 2013; López and Fernández, 2006).

The HRL_4 and lactate clearance did not show any tendency, confirming that the aerobic capacity during exercise and the activation of the BL clearance mechanisms are independent, and that, therefore, they are characteristics that need to be promoted objectively through training.

Regarding BL clearance, it is important to note that decreased renal perfusion also affects lactate elimination (Henderson, 2013). Profuse sweating was noted in the study animals at the end of the test, indicating that they experienced dehydration and possibly decreased renal perfusion.

The recommended way to calculate HRL_4 is promoting an increase in intensity and a BL concentration greater than 4 mmol/L(3). However, some animals will not achieve this due to their high aerobic capacity or efficient clearance mechanisms, making the calculation of HRL_4 difficult to define.

The pauses that are added to the scheme of a field exercise test both in the field and on the treadmill, are discussed, since they can promote the activation of lactate removal mechanisms and at the end do not show the more faithful production of this analyte. For this reason, some authors recommend measuring BL at rest and at the end of the test without taking pauses between steps (Allen et al., 2016).

The standardization of a field exercise test for a particular discipline is useful for the evaluation of disorders induced by exercise (Allen et al., 2016), since it allows knowing the behavior of the performance indices in a healthy horse and to compare it with a non-healthy one.

Given the limited number of animals included in the present study, it is not possible to infer about the results in the CPH population. However, it is a starting point for the construction of breed-specific sporting parameters.

In conclusion, the field exercise test is useful to assess heart rate and BL changes in acute response to exercise in CPHs. It would be useful to evaluate training kinetics with other parameters including blood count and muscle enzymes. To the best of our knowledge, this is the first report on the athletic performance using metabolic parameters such us HRL_4 in paso horses, suggesting it as a potential indicator of conditioning and evolution of physiological adaptations to training evaluating the same animal compared with itself.

Conflict of interests

The authors declare that they have no conflict of interest.

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References

Allen, K.J., Van, Erck Westergren, Franklin, S.H., 2016. Exercise testing in the equine athlete. Equine Vet. Educ. 28, 89–98. http://doi.wiley.com/10.1111/eve.12410.

Arias, M.P., Maya, J.S., Arango, L., 2019. Efectos de dos protocolos de entrenamiento sobre el lactato sanguíneo en caballos de paso fino. Rev. Fac. Med. Vet. Zootec. 66, 219–30. https://revistas.unal.edu.co/index.php/remevez/article/view/84259

Arias, M.P., Sánchez, H.E., Duque, E.C., Maya, L.A., Becerra, J.Z., 2006. Estimación de la intensidad de trabajo en un grupo de caballos criollos colombianos de diferentes andares. CES Med. Vet. Zootec. 1, 18–32. https://revistas.ces.edu.co/index.php/mvz/article/view/213

Boffi, F., 2007. Fisiología del ejercicio en equinos. First ed. Intermédica, Buenos Aires, Argentina.

Capacho, B.D., Arias, M.P. Comparison of lactate and blood chemical analysis in trained and untrained colombian creole horses during a field test. Universidad CES; 2019.

Couroucé, A., Chrétien, M., Valette, J.P., 2002. Physiological variables measured under field conditions according to age and state of training in French Trotters. Equine Vet. J. 34, 91-97. https://doi.org/10.2746/042516402776181141

Enríquez, V., Granados, S., Arias, M.P., Calderón, J.C., 2015. Muscle Fiber Types of Gluteus Medius in the Colombian Creole Horse. J. Equine Vet. Sci. 35, 524–30.

Evans, D.L., 2007. Physiology of equine performance and associated tests of function. Equine Vet. J. 39, 373–83. http://doi.wiley.com/10.2746/042516407X206418

Fortier, J., Goachet, A.G., Julliand, V., Deley, G., 2015. Effects of two field continuous incremental tests on cardiorespiratory responses in Standardbred trotters. J. Anim. Physiol. Anim. Nutr. 99, 244–50. http://doi.wiley.com/10.1111/jpn.12242

Fraipont, A., Van, Erck E., Ramery, E., Fortier. G., Lekeux, P., Art, T., 2012. Assessing fitness in endurance horses. Can. Vet. J. 53, 311–4.

Henderson, I.S., 2013. Diagnostic and prognostic use of L-lactate measurement in equine practice. Equine Vet. Educ. 25, 468–75. http://doi.wiley.com/10.1111/eve.12033

Henneke, D.R., Potter, G.D., Kreider, J.L., Yeates, B.F. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15, 371–2. https://beva.onlinelibrary.wiley.com/doi/full/10.1111/j.2042-3306.1983.tb01826.x

Hodgson, D., McGowan, C., McKeever, K., 2014. The athletic horse. Second ed. Elsevier, Sidney, Australia.

Holdridge, L.R., Grenke, W.C., 1971. Forest environments in tropical life zones: a pilot study, first ed. Pergamon Pr.

Kupczyński, R., Śpitalniak, K., Zwyrzykowska-Wodzińska, A., Soroko, M., 2018. The influence of different workload trainings on some blood parameters in show jumping horses. Vet. Arh. 88, 279–93. http://doi.10.24099/vet.arhiv.170513

Lindner, A., Esser, M., López, R., Boffi, F., 2020. Relationship between resting and recovery heart rate in horses. Animals. 10, 1-8. doi: 10.3390/ani10010120

López, Chicharro, J.L., Fernández, Vaquero, A., 2006. Fisiología del ejercicio. Third ed. Ed. Médica Panamericana, Colombia.

Mann, T., Lamberts, R.P., Lambert, M.I., 2013. Methods of prescribing relative exercise intensity: Physiological and practical considerations. Sports Med. 43, 613-625. https://link.springer.com/article/10.1007/s40279-013-0045-x

McBride, S.D., Mills, D.S., 2012. Psychological factors affecting equine performance. BMC Vet. Res. 8 p. 180. http://bmcvetres.biomedcentral.com/articles/10.1186/1746-6148-8-180

Munsters, C., Van Iwaarden, A., Van Weeren, R., Sloet Van, O., 2014. Exercise testing in Warmblood sport horses under field conditions. Vet. J. 202, 11–9.

Nagy, A., Murray, J.K., Dyson, S.J., 2014. Descriptive epidemiology and risk factors for eliminations from Fédération Equestre Internationale endurance rides due to lameness and metabolic reasons (2008-2011). Equine Vet. J. 46, 38–44. https://pubmed.ncbi.nlm.nih.gov/23574513/

Neto, P.G., Espinosa, O.O., 2009. Determinación de frecuencia cardiaca, frecuencia respiratoria, lactato deshidrogenasa, creatinkinasa y ácido láctico en caballos durante competencia de salto en la Sabana de Bogotá. Rev. Med. Vet. 1, 17. https://ciencia.lasalle.edu.co/mv/vol1/iss17/3

Novoa-Bravo, M., Bernal-Pinilla, E., García, L.F., 2021. Microevolution operating in domestic animals: evidence from the Colombian Paso horses. Mamm Biol 101, 181-192. https://doi.org/10.1007/s42991-021-00103-8

Palacio, B.J., De la Osa, de Vivero R.A, Arias, Gutiérrez. M.P., Zuluaga Araque D.A., Gómez, Restrepo, F.A. Determinación de algunos parámetros hematológicos en caballos criollos colombianos durante una cabalgata de larga duración en Antioquia. Universidad CES; 2013.

Piccione, G., Giannetto, C., Fazio, F., Di Mauro, S., Caola, G., 2007. Haematological response to different workload in jumper horses. Bulg. J. Vet. Med. 10, 21–8.

Young, L.E., Marlin, D.J., Deaton, C., Brown, F., Roberts, C.A., Wood, J.L., 2002. Heart size estimated by echocardiography correlates with maximal oxygen uptake. Equine Vet. J. 34, 467–71. http://doi.wiley.com/10.1111/j.2042-3306.2002.tb05467.x

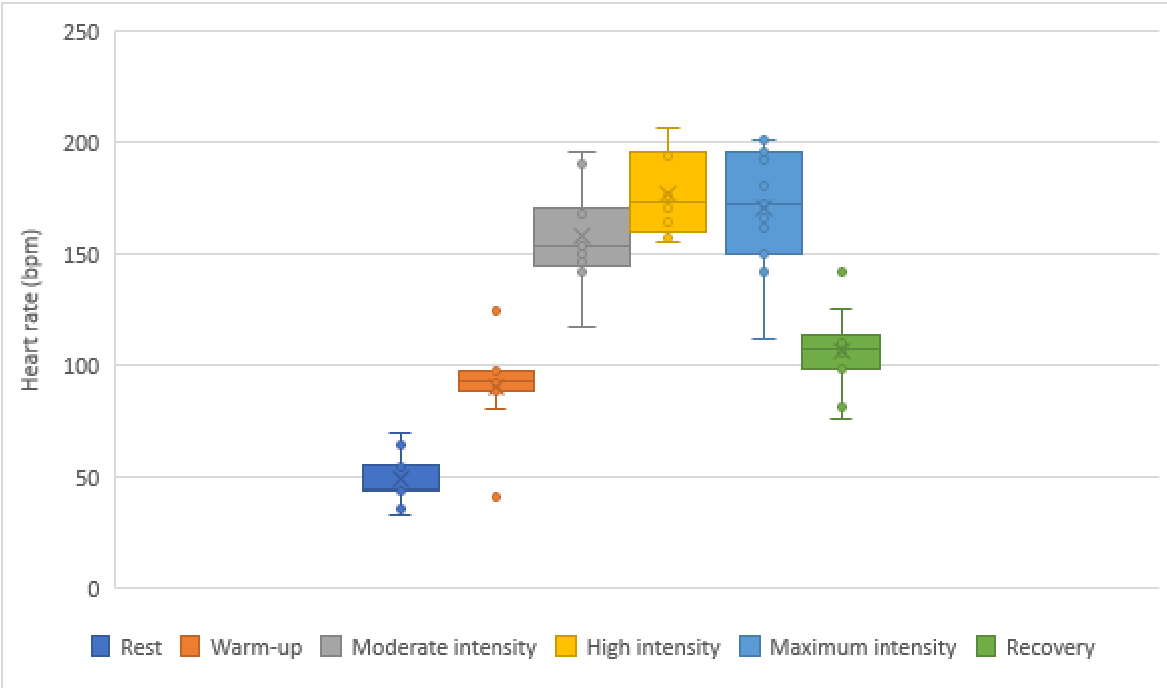

Figure 1. Distribution of heart rate values obtained at each step of the field exercise test used in the Colombian paso horses of study.

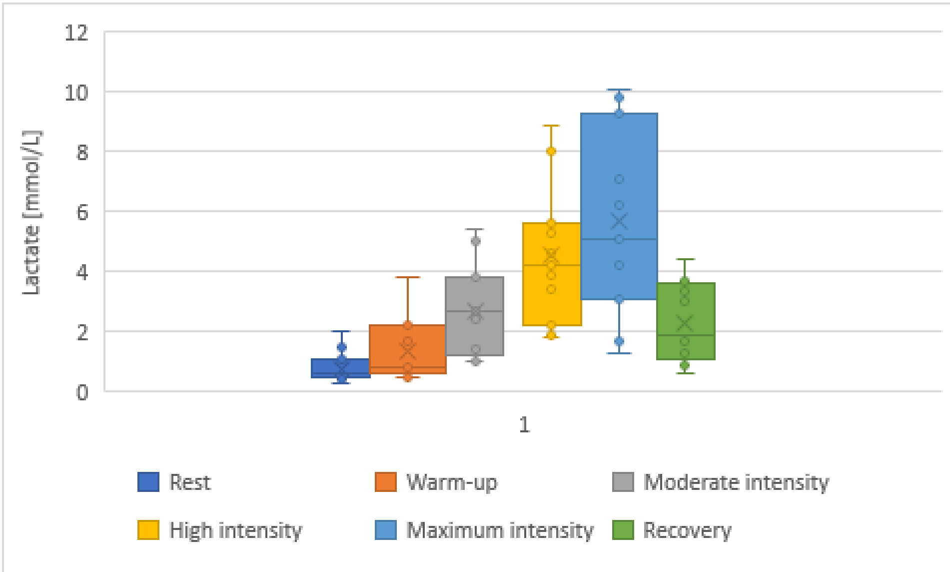

Figure 2. Distribution of blood lactate values obtained at each step of the field exercise test used in the Colombian paso horses of study.

Table 1. Scheme of the field exercise test used in the Colombian paso horses of study.

	Initial heart rate	Warm-up	Moderate intensity	High intensity	Maximum intensity	Recovery
Intensity (% maximum heart rate)	<50	58 to 65	65 to 75	75 to 85	≥ 85	**
Length (in minutes)	ł	15	5	5	5	10

** = Heart rate 10 minutes after the end of the test, walking the animal; ł = Length of the preparation process of the specimen for testing.

Table 2. Heart rate and blood lactate results obtained at each step of the field exercise test used in the Colombian paso horses of study.

Step	**Heart rate (bpm)**			**Blood lactate (mmol/L)**		
	Median (IQR)	SD	CV	Median (IQR)	SD	CV
Initial (<50% of the maximum HR)	45 (44 – 54)	11.3	0.23	0.6 (0.5 – 1.1)	0.53	0.67
Warm-up (58 to 65% of the maximum HR)	93 (88 – 97)	19.6	0.22	0.8 (0.6 – 2.2)	1.06	0.78
Moderate intensity (65 to 75% of the maximum HR)	154 (145 – 171)	22.5	0.14	2.7 (1.2 – 3.8)	1.54	0.57
High intensity (75 to 85% of the maximum HR)	173 (160 – 196)	18.0	0.10	4.2 (2.2 – 5.6)	2.33	0.52
Maximum intensity (≥ 85% of the maximum HR)	172 (150 – 192)	28.0	0.16	5.1 (3.1 – 9.3)	3.13	0.55
Recovery	107 (98 – 110)	18.3	0.17	1.9 (1.1 – 3.6)	1.32	0.57

IQR = Interquartile range, SD = Standard deviation, CV = Coefficient of variation.

Table 3. Heart rate to reach the anaerobic threshold and percentage of blood lactate clearance in the Colombian paso horses of study (presented individually).

Animal	HRL_4 (bpm)	Blood lactate clearance (%)
Horse 1	223	58
Horse 2	178	58
Horse 3	132	56
Horse 4	160	60
Horse 5	140	63
Horse 6	215	63
Horse 7	168	22
Horse 8	184	69
Horse 9	251	65
Horse 10	165	74
Horse 11	205	31

Capítulo 2. Evaluación de grasa corporal en caballos colombianos de Paso: Puntuación de condición corporal y mediciones morfométricas y ecográficas

El presente capítulo está compuesto por un artículo publicado en *Brazilian Journal of Veterinary Research and Animal Science* (Q3).

Durante el desarrollo del experimento se encontró que no existía una técnica que permitiera evaluar objetivamente el porcentaje de grasa corporal de un caballo de paso colombiano. De este modo, no era posible saber si la natación producía cambios sobre la grasa corporal, a menos que se validaran técnicas utilizadas en otras razas de caballos, sobre los animales del estudio. Por lo anterior, se realizó la validación de la técnica por ultrasonido, y los resultados fueron publicados.

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Body fat evaluation in Colombian Paso horses: body condition score and morphometric and ultrasound measurements.

Este artículo fue publicado en Brazilian Journal of Veterinary Research and Animal Science (Q3).

Vínculo del artículo: https://www.revistas.usp.br/bjvras/article/view/171082

Guías para los autores: https://www.revistas.usp.br/bjvras/about/submissions

Nota: Debido a que en algunas tablas se detectó un error de digitación en los valores de "porcentaje de grasa corporal", se solicitó corrección a la revista y consecuentemente, se construyó un *erratum* (archivo anexo).

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FULL ARTICLE

Body fat evaluation in Colombian Paso horses: Body condition score and morphometric and ultrasound measurements

Avaliação da gordura corporal em equinos Paso colombianos: Escore de condição corporal e medidas morfométricas e ultrassonográficas

ABSTRACT

The body condition score (BCS) has been shown to be insufficient in determining the amount of body fat in horses, thus defining obesity. Measurement of the subcutaneous fat thickness (SFT) by ultrasonography should be considered as an appropriate method in the definition of fat distribution at different body locations in horses. Therefore, this study aimed to 1) characterize the SFT in three different anatomical locations (i.e. neck, lumbar region, and gluteal region), 2) evaluate the relationship between BCS and SFT, 3) determine the influence of gender, weight, age, and gait on BCS and SFT measurements, and, 4) explore the agreement between the morphometric measurements [i.e. body mass index (BMI), girth circumference:height at withers ratio (GC:HW), neck circumference:height at withers ratio (NC:HW)], and BCS and SFT in a population of Colombian Paso Horses (CPHs). The Henneke´s body condition scoring was applied to 69 adult CPHs, selected using a convenience sampling. Additionally, BMI, GC:HW, and NC:HW were calculated. Body fat percentage (BF%) was calculated by ultrasound measurement of the SFT in the neck, lumbar region, and gluteal region. The BF% in the CPHs was 41 ± 11.2. The GC:HW, NC:HW, and BMI were not predictors of the BF% or BCS, and neither gender nor gait was decisive in the definition of fattening in the animals of study, although age and weight were determining variables. According to our results, ultrasound is an adequate tool to calculate the BF% of the CPHs. However, it must be accompanied by the Henneke´s BCS assessment.
Keywords: adiposity, body fat, Paso-horse, ultrasound.

RESUMO

O escore de condição corporal (ECC) demonstrou ser insuficiente na determinação da quantidade de gordura corporal em cavalos, definindo a obesidade. A medição da espessura da grassa subcutânea (EGS) por ultrassonografia deve ser considerada como um método apropriado na definição da distribuição de gordura em diferentes locais do corpo em cavalos. Portanto, este estudo teve como objetivo 1) caracterizar a SFT em três diferentes localizações anatômicas (pescoço, região lombar e região glútea), 2) avaliar a relação entre ECC e EGS, 3) determinar a influência de gênero, peso, idade, e marcha nas medidas ECC e EGS e, 4) explorar a concordância entre as medidas morfométricas [i.e. índice de massa corporal (IMC), razão do circunferência da cintura:altura da cernelha (CC:AC), razão do circunferência do pescoço:altura da cernelha (CP:AC)], ECC e EGS em uma população de Cavalos Paso colombiano (CPCs). A pontuação da condição corporal de Henneke foi aplicada a 69 CPCs adultas, selecionadas por amostragem de conveniência. Além disso, IMC, CC:AC e CP:AC foram calculados. O percentual de grassa corporal (%GC) foi calculado por meio da ultrassonografia da EGS no pescoço, região lombar e região glútea. O %GC nas CPCs foi de 6,4 ± 1,1. O CC:AC, CP:AC e IMC não foram preditores da %GC ou ECC, e nem o gênero nem a marcha foram decisivos na definição de obesidade nos animais do estudo, embora a idade e o peso fossem variáveis determinantes. De acordo com nossos resultados, o ultrassom é uma ferramenta adequada para calcular o %GC das CPCs. No entanto, deve ser acompanhado pela avaliação do ECC do Henneke.

Palavras-chave: adiposidade, grassa corporal, cavalo Paso, ultrassom.

Introduction

The body condition score (BCS) has been shown to be insufficient in determining the amount of body fat in horses and, therefore, in the definition of obesity (DUGDALE et al., 2011a; ARGO et al., 2012). Some studies in obesity-predisposed horses have determined that the Henneke´s horse BCS (HENNEKE et al., 1983) should be adjusted according to the morphology of each breed, given the differences found in regional adiposity and in the general appearance of the animals (MARTIN-GIMÉNEZ et al., 2016a, 2016b). The Henneke´s BCS is an indirect method of body fat scoring that presents difficulties in differentiating an overweight and an obese horse (PEARSON et al., 2018) since it is not directly related to the amount of subcutaneous, cavitary, and visceral adipose tissue. Furthermore, to the authors' experience it does not allow to determine the nutritional status of the horse.

Defining obesity in athletic horses is a priority. Obesity is a restrictive condition for sport, due to the metabolic disorders that occur and the risk of laminitis. Additionally, it decreases the body's ability to develop functional muscle mass, necessary when performing sports activities, predisposing the obese horse to exercise-induced pathologies. On the other hand, obesity has been linked to inflammatory changes at the articular level by biomarkers such as prostaglandin E in synovial fluid (PEARSON et al., 2018). This demonstrates that the accumulation of adipose tissue implies deleterious changes in horses' health.

Obesity has been traditionally evaluated through the Henneke´s BCS, nuchal crest, and body mass index (BMI; THATCHER et al., 2008). However, it is complex to determine obesity based on visual methods in obesity-predisposed and muscular breeds, or subtypes of the same breed (e.g. gait). Establishing objective body fat measurements is essential to avoid the overestimation of obesity in these breeds and individuals. Furthermore, a better understanding of fat deposition could help to understand the dynamics of lipid disorders in the horse. Consequently, body fat-objective indicators are needed to increase the precision of the assessment of body composition to highlight animals at risk of obesity-related diseases.

Measurement of the subcutaneous fat thickness (SFT) by ultrasonography has been considered as an appropriate method when defining the subcutaneous distribution of adipose tissue in different body locations and as an appropriate method when monitoring the increase in subcutaneous adipose tissue in horses with phenotypically established deposition patterns, as many Andalusian horse-related breeds (MARTIN-GIMÉNEZ et al., 2016b). This method has also been used in young animals, in order to monitor body development during the lactation period (HUNKA et al., 2014). WESTERVELT et al. (1976) described a high correlation between body fat percentage (BF%) —measured by ultrasound of the gluteal region, and the amount of fat tissue extracted from the carcasses of horses and ponies ($r^2 = 0.93$), validating this method.

Additional *ante-mortem* approaches have been reported in horses to estimate general adiposity. CARTER et al. (2009) determined that girth circumference:height at withers ratio (GC:HW) is the most appropriate indirect measure for the evaluation of general adiposity, and that neck circumference:height at withers ratio (NC:HW) is an adequate measure in the evaluation of regional adiposity of the neck. These morphometric measurements have also been used in Icelandic horses (JENSEN et al., 2016), Argentinean saddle bred crossbreed and Paso horses (VELÁSQUEZ-MOSQUERA et al., 2016), and Portuguese Asinina de Miranda donkey breed (QUARESMA et al., 2013). By dissecting white fat in ponies carcasses, DUGDALE et al. (2011) confirmed that GC:HW measurement and ultrasound of retroperitoneal adipose tissue have a high predictive capacity for general body adiposity in the species. Nevertheless, to the author´s knowledge no studies on SFT in Colombian Paso horses (CPHs) are available so far in Colombia or elsewhere.

Therefore, this study aimed to 1) characterize the SFT in three different anatomical locations (i.e. neck, lumbar region, and gluteal region), 2) evaluate the relationship between BCS and SFT, 3) determine the influence of gender, weight, age, and gait on BCS and SFT measurements, and, 4) explore the agreement between the morphometric measurements (i.e. body mass index —BMI, GC:HW, NC:HW), and BCS and SFT in a population of Colombian Paso Horses (CPHs).

Materials and Methods

Animals

Sixty-nine stabled adult CPHs were selected using a convenience sampling. Animals were located in the Eastern sub-region of the Province of Antioquia (Colombia). Thirty-nine females and 30 male horses, of ages of 2-22 years, composed the study group. Table 1 presents an additional gait differentiation for the characterization of the study population.

Body condition score assessment

A veterinarian trained for the task, assessed the BCS to the whole study population, considering the Henneke´s body scoring (HENNEKE et al., 1983) and Carter's nuchal crest scorings (CARTER et al., 2009), with a “blind” assessment of the measurements taken by ultrasound.

Ultrasound measurements

In order to describe a possible method to be applied in the field, animals were not shaved, then, measurements were taken using antiseptic alcohol on clean hair. Ultrasonographic images were taken at three defined points, according to previous reports in other breeds (MARTIN-GIMÉNEZ et al., 2018). Anatomic landmarks were identified by palpation and visual assessment, as follows: 1) SFT-75% of neck length (from the base of the ear towards the dorsal edge of the scapula, in a straight line in the anatomical demarcation formed by the trapezius muscle and the nuchal ligament laminar portion); 2) SFT-lumbar region (3-cm laterally, at the L6); and, 3) SFT-gluteal region (50% of the path between the coxal tuberosity and the greater trochanter of the femur and 5 cm in the direction of the sacrum). All measurements were taken in triplicate, calculating the coefficient of variation (CV) among measurements to guarantee mean homogeneity and representativeness (CV ≤ 20%). The Sonoscape® A5V (SonoScape Medical Corp. China) equipment was considered, using a 7-8 MhZ linear probe to measure the SFT at the neck, lumbar region and gluteal points, and a 3.5-4.5 MhZ convex probe for the gluteal muscle. All images were taken in B-mode and under the image freezing function, using the place with the highest SFT. The ultrasonographic technique was subjected to a pre-test on animals other than the study ones, to verify repeatability, reproducibility, and practicality of the method.

Morphometric measurements

Weight was measured using a weight tape for horses, as previously reported (JENSEN et al., 2016). The height at the withers was measured with the horse in pedestation, locating each animal with the fore and hind limbs perpendicular to a flat floor (MARTINSON et al., 2014). The perimeter of the thorax and the circumference of the neck were taken completely surrounding the reference points (cross and the most prominent portion of the neck, respectively). Chest width was measured as the length between the scapulo-humeral joints, and the body length resulted from the measurement between the scapulo-humeral joint and the ipsilateral ischial tuberosity. All morphometric values were obtained using a conventional tape measure. The BMI was calculated according to CARTER et al. (2009) [weight (kg)/height at the withers (m^2)]; and, NC:HW ratio [neck circumference (cm)/height at the withers (m^2)] and GC:HW ratio [thorax perimeter (cm)/height at the withers (m^2)] according to Jensen et al. (2016). The BF% was calculated according to the formula established by KANE et al. (1987) [5.47 × SFT-gluteal region (cm) + 2.47].

Statistical analysis

The data was collected in Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and then exported to Stata 16.0 (StataCorp, 2020, College Station, Texas, USA) for analysis. Descriptive statistics (i.e. mean, standard deviation, minimum value, maximum value) were defined for all continuous variables. In addition, a bivariate Pearson correlation analysis was performed between all variables. Since the data obtained behaved in a normal way according to Shapiro-Wilk test (p=0.0913), the one-way ANOVA analysis of variance was performed to compare the means between the SFT values at the three anatomical points and Henneke´s BCS. All analyses considered a significance level of $p \leq 0.05$.

Results

According to the selection of the animals of study, distribution by gender, and gait were defined (Table 1). According to age, 27 horses were between 2 and 5 years, 33 were between 6 and 10 years, and 9 horses were >10. Morphometric and ultrasound measurements were compiled, according to the values obtained from all the animals in the study (Table 2).

Once the horses were classified according to Henneke´s BCS, the circumference of the neck, ultrasound measurements, BF%, and BMI were grouped by means and standard deviation (Table 3).

Neck circumference, BF%, and BMI were found to be notoriously different when the Henneke´s BCS was equal to or greater than 7/9, whereas that changes between Henneke´s BCS of 7/9 and 8/9 were not easily distinguishable. This pattern was repeated for Henneke´s BCS of 5/9 and 6/9 (Table 3). The behavior of the GC:HW ratio according to Henneke´s BCS, showed a progressive and uniform increase in relation to the BCS (low SD). In contrast, the NC:HW ratio showed no notable changes in the 5/9 score (Table 4).

When the results of GC:HW, NC:HW, and BF%, according to gait and gender were obtained, it was found that GC:HW in females was greater than or equal to the same indicator in males, independent of their gait. In contrast, the NC:HW was higher for males than for females in all gaits. Additionally, BF% did not show a specific trend according to gait or gender (Table 5), nor did it seem to correspond to the GC:HW or NC:HW ratios.

According to the bivariate Pearson analysis ($p \leq 0.05$), a correlation between the Henneke´s BCS, BMI, and BF%, and the morphometric and ultrasound measurements was found (Table 6).

Since the age of the horses of the study was found to be positively correlated with BF% (r=0.90; p=0.001), the respective information was distributed according to the relevant age groups (youth —2 to 6 years, adults —6 to 10 years, and seniles —> 10 years (Table 7). Similarly, since the weight was found to be positively correlated with BF% (r=0.82; p=0.038), the corresponding information is presented according to the groups of relevance (Table 8). Table 9 shows the non-significant results found in ultrasound measurements and BF% according to gender.

Discussion

Ultrasonography has become an important and objective method for the evaluation of regional fat distribution since it allows independent estimation of the subcutaneous adipose tissue. This method is technically limited in areas with large amounts of connective tissue within adipose tissue (e.g. nuchal crest), because there is a very discrete limit that separates adipose tissue and muscle, affecting the ultrasound reading (MARTIN-GIMÉNEZ et al., 2016a). This situation was confirmed in the present study, since an important number of horses of the study population (n=35) showed Carter's nuchal crest scoring of 4 and 5, making it difficult to carry out the measurements in this anatomical area. It is important to consider that ultrasonographic image contrast between body tissues is essential and must be guaranteed through the frequency of the transducer, as well as, the selection of an anatomical location, where it is feasible to discern the density of each tissue layer. In the present study, it was determined that the lumbar region and gluteal muscle SFT measurements were the most appropriate locations for ultrasonographic evaluation in CPHs. On the other hand, from the practical point of view, it was shown that this kind of ultrasound does not require sedation, in addition to the fact that it is not necessary to shave the animal´s skin or to use difficult-to-clean substances such as ultrasound gel.

JENSEN et al. (2016) found that neither age nor gender had an effect on BCS or on associated morphometric measures. In the present study, this finding was also found for gender. However, it was also established that age was positively correlated with BF% (p = 0.001), within >10 years-old animals included in the present study —an obesity-predisposed age group, meanwhile, gait did not influence the results.

Our findings indicated that weight was positively correlated with BF% (p = 0.038). It seems that CPHs exceeding 351 kg of BW are mostly prone to deposit adipose tissue regardless of their gait. This effect may be due to the morphological characteristics of these animals with respect to other breeds of saddle horses since they are usually small and in turn do not have the body capacity to cumulate a greater component of muscle mass. It is important to define further study approaches on gait effect since morphologically, the appearance of the individual can change from one to another (e.g. different height and neck size) for the breed object of this study.

The Henneke´s BCS is subjective by default and may result in underestimation of the horse's condition when the person who values it is not an expert, as verified by JENSEN et al. (2016) in a study on 254 Islamic horses. Other ratios such as GC:HW and NC:HW should not be applied interchangeably in all breeds since racial morphometric differences and those due to gender dimorphism could modify the results. Additionally, despite the fact that both increased with Henneke´s BCS, when the category corresponded to 7/9, 8/9, or 9/9 they were not differentiable, making it impossible to discriminate between overweight and obese animals.

According to previous studies, a horse or pony is considered over-weighted (Henneke´s BCS ≥ 7/9) when the GC:HW is ≥ 1.26 and ≥ 1.33, respectively. While the NC:HW is used, a horse or pony is considered obese when the value is ≥ 0.63 and ≥ 0.68, respectively (CARTER et al., 2009; JENSEN et al., 2016). However, this assessment was not the same for the CPHs on the present study, since the NC:HW was higher than 1.26 when the Henneke´s BCS was ≥ 3/9 and, on the contrary, the GC:HW was lower than 1.20 in all the Henneke´s BCS categories. Despite the fact that some studies have determined that all chest circumference-related morphometric measures are highly associated to Henneke´s BCS (DONALDSON et al., 2004), the result of such study highlighted that the morphometric differences between breeds do not allow to transfer all the methods for body fat evaluation, without being previously validated. Additionally, gender dimorphism in the horse involves changes in neck circumference and size of the animal; therefore, it must be taken into account when using a method in horses, independent of gender.

The BMI mean found in the present study was 183.98 kg/m^2, which is lower than the mean index found in ponies (212.14 kg/m^2; DUGDALE et al., 2011a) and to the mean found in athletic horses (215 kg/m^2; THATCHER et al., 2008). This index is not very sensitive to determine body composition since it can only indicate the amount of mass per unit area and differs strongly between horse breeds. As evidenced in the results of the present study, BMI appears differentiable in the extreme categories of Henneke´s BCS and BF%, so it is not a sensitive indicator to determine adiposity in CPHs.

A study by SILVA et al. (2016) determined that SFT measurement on the back at the level of the L3 was correlated with body condition in donkeys and horses, with a correlation coefficient greater than 0.7. In contrast, the present study found that SFT on the lumbar area is correlated to BF% (p = 0.000), despite not being reflected on the Henneke´s BCS results. On the other hand, QUARESMA et al. (2013) reported a positive correlation between the SFT measurement on the back, ribs, and haunch and Henneke´s BCS and, therefore, both methods should be used to define obesity in donkeys. Similar findings are reported herein, where Henneke´s BCS rating was found to be an approximation tool in the definition of body composition, which in turn must be carried out in detail to determine the degree of body adiposity.

MANSO-FILHO et al. (2009) used a formula developed by WESTERVELT et al. (1976), considering the same anatomical reference point in the gluteal region. However, the constant values differ from those presented in this study. The formula used to estimate the BF% was the result of the comparison between the ultrasound measurement of the gluteal muscle and the total ether extractable body fat carried out on pony carcasses ($r^2 = 0.93$). By this calculation, the BF% appears to be higher than the one calculated using the formula of KANE et al. (1987). It should be considered that, by the time both methods were developed, the resolution of the ultrasound image was not high and allowed errors in the SFT measurement. Compared with other similar studies, the calculation proposed by KANE et al. (1987) seems to underestimate the BF% (MANSO-FILHO et al., 2009; Silva et al., 2016).

MANSO-FILHO et al. (2009) found that stallions resulted in a higher BF% (16.63 ± 1.24) compared to non-pregnant mares (13.95 ± 0.92) and to foals under 18 months (10.38 ± 0.26). In addition, the type of physical activity performed by the animals represented significant differences in BF% when dairy- was compared with show-working (10.82 vs. 14.77%, respectively). On studies on thoroughbred foals and fillies up to 2 years of age, females were found to contain fatter tissue than males (GEE et al., 2003). However, when compared to the results of the present study, no gender-related differences in BF% were found. This result was perhaps due to the differences between the age groups within each group (females and males).

It is interesting to explore other methods that do not require the use of any tool other than the conventional tape measure, as the study by POTTER et al. (2015), where it was possible to obtain a modified Henneke´s BCS (named *body score index*), using the following formula: BCI = [chest circumference$^{0.5}$ + abdomen perimeter + neck circumference$^{1.2}$)/height$^{1.05}$)]. This calculation was correlated with BF%, measured using the deuterium dilution method ($r^2 = 0.772$). Such method was already validated by DUGDALE et al. (2011b), with a high correlation level, using the total white adipose tissue in cadavers ($r^2 = 0.97$). GEE et al. (2003) also found an important correlation between adipose tissue predicted by ultrasound in live thoroughbred foals and fillies and adipose tissue effectively dissected *post-mortem* ($p = 0.002$).

Despite being discussed in multiple studies, Henneke´s BCS is a complementary visual tool that can facilitate the assessment of obese animals. In a study conducted in a group of mares with extreme BCS (very thin vs. obese), it was determined that Fat tissue thickness measured by ultrasound in the back, ribs, and neck was correlated with Henneke´s BCS results. This was given to each animal, demonstrating that, for the most extreme scores on the scale, it is feasible to determine adipose tissue deposition. Furthermore, they found that the most sensitive location is the base of the tail (GENTRY et al., 2004). From this information and the results of the present study, it can be inferred that both the measurement of adipose tissue by ultrasound and Henneke´s BCS assessment, age and weight are useful complementary variables for the evaluation of the body's adiposity in CPHs.

Conclusion

From a general point of view, the assessment of the body composition in the horse must involve a set of non-invasive methods, obtaining the greatest amount of data related to the deposition of adipose tissue. This is important since it has been shown that fat deposition patterns can be different, depending on the anatomical location, physical activity, and genetic predisposition of the animal. According to our results, the evaluation of the body composition in the CPHs must be composed by the Henneke´s BCS assessment (with special emphasis on the base of the tail), SFT-lumbar region and/or SFT-gluteal muscle measurement, determination of the BF%, and differentiation by age and gender. The regional adiposity of the neck should be explored independently since it was not found as a determining factor in the evaluation of overweight or obesity in CPHs in the present study.

Conflict of Interest

The authors state that they have no conflicts of interest to declare.

Ethics Statement

The Ethics Committee for Animal Experimentation of the Universidad de Antioquia (Medellín, Colombia) approved all the procedures performed on the animals of the present study (Act #122, February 5th, 2018).

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References

Argo CM, Curtis GC, Grove-White D, Dugdale AHA, Barfoot CF, Harris PA. Weight loss resistance: A further consideration for the nutritional management of obese Equidae. Vet J. 2012;194:179–88. https://doi.org/1016/j.tvjl.2012.09.020

Carter RA, Geor RJ, Burton Staniar W, Cubitt TA, Harris PA. Apparent adiposity assessed by standardized scoring systems and morphometric measurements in horses and ponies. Vet J. 2009;179(2):204–10. https://doi.org/10.1016/j.tvjl.2008.02.029

Donaldson MT, McFarlane D, Jorgensen AJ, Beech J. Correlation between plasma a-melanocyte-stimulating hormone concentration and body mass index in healthy horses. Am J. Vet. Res. 2004;65(11):1469–73. https://doi.org/10.2460/ajvr.2004.65.1469

Dugdale AH, Curtis GC, Harris PA, Argo CM. Assessment of body fat in the pony: part I. Relationships between the anatomical distribution of adipose tissue, body composition and body condition. Equine Vet. J. 2011a;43(5):552–61. https://doi.org/10.1111/j.2042-3306.2010.00330.x

Dugdale AH, Curtis GC, Milne E, Harris PA, Argo C. Assessment of body fat in the pony: Part II. Validation of the deuterium oxide dilution technique for the measurement of body fat. Equine Vet J. 2011b;43(5):562–70. https://doi.org/10.1111/j.2042-3306.2010.00327.x

Gee E, Fennessy P, Morel P, Grace N, Firth E, Mogg T. Chemical body composition of 20 Thoroughbred foals at 160 days of age, and preliminary investigation of techniques used to predict body fatness. N Z. Vet. J. 203;51(3):125–31. https://doi.org/10.1080/00480169.2003.36351

Gentry LR, Thompson DL, Gentry GT, Del Vecchio RP, Davis KA, Del Vecchio PM. The relationship between body condition score and ultrasonic fat measurements in mares of high versus low body condition. J Equine Vet. Sci. 2004;24(5):198–203. https://doi.org/10.1016/j.jevs.2004.04.009

Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 1983;15:371–2. https://doi.org/10.1111/j.2042-3306.1983.tb01826.x

Hunka M, Cordeiro Manso H, Bernardo R, da Silva E, Ferreira L, Manso-Filho H. Development and body composition of Quarter horse foals during nursing. Open J. Vet. Med. 2014;4:276–80. https://doi.org/10.4236/ojvm.2014.411033

Jensen RB, Danielsen SH, Tauson AH. Body condition score, morphometric measurements and estimation of body weight in mature Icelandic horses in Denmark. Acta Vet. Scand. 2016;58(1):59. https://doi.org/10.1186/s13028-016-0240-5

Kane RA, Fisher M, Parrett D, Lawrence LM. Estimating fatness in horses. In Proceedings of the 10th Equine Nutrition and Physiology Symposium. 1987; 127. p. 31.

Manso-Filho HC, Cordeiro Manso H, Ferreira LMC, Santiago TA, Wanderley EK, Abreu JMG. Percentagem de gordura de cavalo criados em região tropical. Acta Scien. Vet. 2009;37(3):239–43. https://doi.org/10.22456/1679-9216.16336

Martin-Giménez T, Aguirre-Pascasio CN, de Blas I. Ultrasonographic assessment of regional fat distribution and its relationship with body condition in an easy keeper horse breed. J. Equine Vet. Sci. 2016a;39:69–75. https://doi.org/10.1016/j.jevs.2016.01.010

Martin-Gimenez T, de Blas I, Aguilera-Tejero E, Diez de Castro E, Aguirre-Pascasio CN. Endocrine, morphometric, and ultrasonographic characterization of neck adiposity in Andalusian horses. T Domest. Anim. Endocrinol. 2016b; 56:57–62. https://doi.org/10.1016/j.domaniend.2016.02.003

Martin-Giménez T, Aguirre-Pascasio CN, De Blas I. Development of an index based on ultrasonographic measurements for the objective appraisal of body condition in Andalusian horses. Span J. Agric. Res. 2018;15(4):6–9. https://doi.org/10.5424/sjar/2017154-11732

Martinson KL, Coleman RC, Rendahl AK, Fang Z, McCue ME. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. J. Anim. Sci. 2014; 92:2220–3. http://dx.doi.org/10.2527/jas.2013-6689

Pearson W, Wood K, Stanley S. MacNicol J. Exploring relationships between body condition score, body fat, activity level and inflammatory biomarkers. J Anim. Physiol. An. N. 2018;102(4):1062–8. http://dx.doi.org/10.1111/jpn.12893

Potter SJ, Harris PA, Bailey SR. Derivation of a new body condition index to estimate body fat percentage from morphometric measurements: Comparison with body condition score. J Equine Vet. Sci. 2015;35:400–17. http://dx.doi.org/10.1016/j.jevs.2015.03.073

Quaresma M, Payan-Carreira R, Silva SR. Relationship between ultrasound measurements of body fat reserves and body condition score in female donkeys. Vet J. 2013;197(2):329–34. http://dx.doi.org/10.1016/j.tvjl.2012.12.031

Silva SR, Payan-Carreira R, Quaresma M, Guedes CM, Santos AS. Relationships between body condition score and ultrasound skin-associated subcutaneous fat depth in equids. Acta Vet. Scand. 2016;58(1):62. http://dx.doi.org/10.1186/s13028-016-0243-2

Thatcher CD, Pleasant RS, Geor RJ, Elvinger F, Negrin KA, Franklin J, Were SR. Prevalence of obesity in mature horses: an equine body condition study. J Anim. Physiol. An. N. 2008;92(2):222. http://dx.doi.org/10.1111/j.1439-0396.2007.00789_8.x

Velásquez-Mosquera JC, Mendoza-Sánchez G, Corrales-Álvarez JD, Parra-Pineda,MA, Medina-RodríguezAC, Izquierdo-Sánchez CD, Gonzales-Acosta JP. Asociación de medidas morfométricas con grasa en el anca en caballos de salto en una escuela ecuestre de Bogotá. Rev Med. Vet. 2016;(32):67–77. http://dx.doi.org/10.19052/mv.3856

Westervelt RG, Stouffer JR, Hintz HF, Schryver HF. Estimating fatness in horses and ponies. J Anim. Sci. 1976;43(4):781–5. https://doi.org/10.2527/jas1976.434781x

Table 1 — Distribution of the study population of Colombian Paso horses, according to gait and gender (n = 69).

Gender/Gait	Trot	Trocha	Paso fino	Total
Female	10	16	13	39
Male	10	11	9	30
Total	20	27	22	69

Table 2 — Morphometric and ultrasound measurements obtained from the study population of Colombian Paso horses (n = 69).

Variable	Mean	Minimum value	Maximum value
Circumference of the neck [cm]	99.1 ± 9.4	61	114
Perimeter of the thorax [cm]	163.7 ± 7.9	138	193
Chest width [cm]	34.2 ± 4.0	24	44
Body length [cm]	154.2 ± 10.9	138	216
Height at the withers [cm]	138.6 ± 3.2	130	148
Weight [kg]	350.7 ± 36.2	268	486
SFT-75% of neck length [mm]	7.5 ± 2.3	2.1	13.4
SFT-lumbar region [mm]	7.0 ± 2.2	3.2	13.2
SFT-gluteal region [mm]	7.1 ± 2.1	3.4	14

Gluteus muscle thickness [mm]	77.3 ± 12.2	42.2	98.9
Body fat percentage	41 ± 11.2	21.3	79.2

SFT: Subcutaneous fat thickness.

Table 3 — Mean and standard deviation (SD±) for ultrasound measurements, circumference of the neck, ultrasound measurements, body fat percentage (BF%), and body mass index (BMI), according to Henneke´s body condition score (BCS) in the study population of Colombian Paso horses (n = 69).

Henneke´s BCS	**Circumference of the neck [cm]**	**SFT-75% of neck length [mm]**[a,ł]	**SFT-lumbar region [mm]**	**SFT-gluteal region [mm]** [b,ł]	**BF%**	**BMI [kg/m^2]**	**n**
3/9	90 ± 11	3.7 ± 2.2	4.0 ± 1.1	4.7 ± 1.8	28 ± 12.7	157 ± 0.0	2
5/9	87 ± 27	6.1 ± 1.5	5.1 ± 0.1	5.7 ± 0.1	34 ± 9.9	171 ± 0.0	2
6/9	93 ± 14	6.6 ± 1.7	6.3 ± 2.0	5.6 ± 1.3	33 ± 11.3	172 ± 11	8
7/9	99 ± 7.3	7.5 ± 2.2	7.2 ± 2.2	7.1 ± 1.8	41 ± 11.4	178 ± 13	32
8/9	103 ± 6.3	8.1 ± 2.3	7.4 ± 2.2	7.9 ± 2.3	46 ± 11.2	194 ± 15	25

BCS: Body condition score; SFT: Subcutaneous fat thickness; BF%: Body fat percentage; BMI: Body mass index. [ł] Statistical significance according to ANOVA analysis ($p \leq 0.05$); [a] p = 0.0042; [b] p = 0.0127.

Table 4 — Morphometric measurements according to Henneke´s body condition score (BCS; mean and SD ±) in the study population of Colombian Paso horses (n = 69).

Henneke´s BCS	GC:HW mean	NC:HW mean
3/9	1.12 ± 0.01	0.63 ± 0.03
5/9	1.15 ± 0.02	0.62 ± 0.18
6/9	1.16 ± 0.03	0.67 ± 0.10
7/9	1.18 ± 0.04	0.72 ± 0.05
8/9	1.20 ± 0.06	0.74 ± 0.04

BCS: Body condition score; GC:HW: Girth circumference:height at withers ratio; NC:HW: Neck circumference:height at withers ratio.

Table 5 — Mean of the calculated values for morphometric measurements, according to gait and gender in the study population of Colombian Paso horses (n = 69).

	GC:HW	NC:HW	BF%
Trot			
Female	1.21	0.71	40
Male	1.16	0.74	46
Trocha			
Female	1.19	0.69	43
Male	1.16	0.73	39
Paso fino			
Female	1.18	0.69	40

Male	1.18	0.76	6.3

GC:HW: Girth circumference:height at withers ratio; NC:HW: Neck circumference:height at withers ratio; BF%: Body fat percentage.

Table 6 —Correlation results between Henneke´s body condition score (BCS), body mass index (BMI) and body fat percentage (BF%), and the morphometric and ultrasound measurements in the study population of Colombian Paso horses (n = 69).

	Categories	***r*-value**	***p*-value***
Henneke´s BCS	Circumference of the neck	0.77	0.012
	Perimeter of the thorax	0.66	0.008
	Height at the withers	0.62	0.050
BMI	NC:HW ratio	0.62	0.045
BF%	Age	0.90	0.001
	Weight	0.82	0.038
	SFT-lumbar region	0.78	0.000
	SFT-gluteal muscle	0.76	0.000

BCS: Body condition score; BMI: Body mass index; NC:HW: Neck circumference:height at withers; BF%: Body fat percentage; SFT: Subcutaneous fat thickness. *Statistical significance according to Pearson analysis ($p \leq 0.05$).

Table 7 — Mean and standard deviation (SD±) of the body fat percentage (BF%) according to the age group in the study population of Colombian Paso horses (n = 69).

Age [years]	**BF% mean**	**Minimum value**	**Maximum value**	**n**
2 to 6	44 ± 11.3	29	66	27

6 to 10	39 ± 11.2	21	79	32
>10	38 ± 11.3	23	60	10

BF%: Body fat percentage.

Table 8 — Mean and standard deviation (SD±) of the body fat percentage (BF%) according to the weight in the study population of Colombian Paso horses (n = 69).

Weight [kg]	**BF% mean**	**Minimum value**	**Maximum value**	**n**
≤ 300	35 ± 4.6	29	43	4
301-350	38 ± 11.2	21	66	38
≥ 351	47 ± 11.4	27	79	27

BF%: Body fat percentage.

Table 9 — Mean and standard deviation (SD±) of the subcutaneous fat thickness (SFT) and of the body fat percentage (BF%) according to gender in the study population of Colombian Paso horses (n = 69).

	Females (n=39)	**Males (n=30)**	***p*-value***
SFT-75% of neck length	6.83 ± 1.93	8.29 ± 2.41	0.164
SFT-lumbar region	7.16 ± 2.27	6.84 ± 2.13	0.481
SFT-gluteal muscle	7.03 ± 2.16	7.21 ± 1.94	0.221
Gluteus muscle thickness	74.53 ± 12.51	80.94 ± 11.06	0.409
BF%	41 ± 11.3	42 ± 11.2	0.541

SFT: Subcutaneous fat thickness; BF%: Body fat percentage. *Statistical significance according to Pearson analysis ($p \leq 0.05$).

Capítulo 3. Conversión de masa corporal y mejor respuesta a la insulina en caballos colombianos de paso (CPH) sometidos a un programa de entrenamiento de natación.

Este capítulo describió los resultados que dan respuesta al objetivo específico 4: *"Evaluar los cambios producidos sobre el espesor del tejido adiposo de un grupo de CCC sometidos a un protocolo de natación".* Los resultados fueron compilados en un manuscrito ACEPTADO para publicación en la revista de divulgación científica Comparative Exercise Physiology (Q3). La siguiente es la última versión del manuscrito según sugerencias de los evaluadores de la revista. Los resultados de este capítulo fueron sometidos y aceptados para su presentación en el XVI Encuentro Nacional y IX Internacional de Investigadores de las Ciencias Pecuarias - ENICIP 2021 (Virtual): "efecto de un protocolo de natación sobre la grasa corporal, hormonas y actividad muscular en caballos de paso colombianos".

Guías para los autores:
https://www.wageningenacademic.com/pb-assets/wagen/files/cep/Guideline%20for%20Authors%20-%20Comparative%20Exercise%20Physiology.pdf

Body mass conversion and improved insulin response in Colombian Paso Horses (CPHs) subjected to a swimming training program.

Body mass and insulin response in horses subjected to swimming.

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Abstract
Background: Overweight and obesity in horses affect their athletic performance negatively, making it therefore necessary to develop training protocols that reduce their body fat without causing hoof injuries. Objectives: To describe the effect of the application of a swimming training program on metabolic and endocrine variables, in addition to evaluating the changes in subcutaneous fat thickness (SFT) in a group of overweight Colombian Paso Horses (CPHs). Methods: Six CPHs were subjected to a decreasing intensity swimming program for four months. The effect of this training on metabolic variables (i.e. aspartate aminotransferase (AST), creatine kinase (CK), triglycerides, cholesterol, glucose) and endocrines (cortisol and insulin) was studied every two months. Additionally, changes in the neck, lumbar and gluteal SFTs were evaluated. The information was analyzed using descriptive statistics, in addition to repeated measures analysis of variance for non-parametric data in the three training moments ($p<0.1$) and correlation analysis between the SFT and the metabolic and endocrine variables of interest.
Results: The swimming training program for CPHs tested in this study produced more evident redistribution of adipose tissue in the gluteal region (Initial SFT= 5.2 ± 2.08 mm; Final SFT= 3.45 ± 2.8 mm), conversion of body mass without weight modification, and use of energy sources such as triglycerides and increased sensitivity to insulin. Main limitations: Horses studied were not selected according to age, nor were control animals used. In addition, the limited number of horses makes extrapolation of the results inappropriate. Conclusions: Swimming training program with decreasing intensity carried out over a four-month period modified body adipose tissue in CPHs, promoting the use of energy sources such as triglycerides and increased insulin sensitivity.

Key words: adipose tissue, EMS, horse, obesity, overweight, training.

Competing interests
The authors have declared no competing interests.

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Introduction
Obesity is defined as the general or localized increase in adipose tissue and is associated with the intake of diets rich in soluble carbohydrates and low energy expenditure, that generate

hypertrophy and hyperplasia of adipocytes (Frank 2011; Reynolds et al, 2019). Increased adipose tissue produces an alteration in the concentration of adipokines, such as leptin and adiponectin (Frank 2011; Moore et al, 2019). Furthermore, obesity leads to a state of chronic inflammation accompanied by sustained oxidative stress, maintaining corticotropin-releasing hormone (CRH) levels high. In addition, there is an activation of the hypothalamic-pituitary-glandular axis that increases the expression of the 11-β hydroxysteroid dehydrogenase type 1 enzyme in adipocytes, which converts cortisone into active cortisol (Espíndola-Antunes and Kater 2007).

Obesity promotes Equine Metabolic Syndrome (EMS), which occurs accompanied by Insulin Dysregulation (ID), characterized by an imbalance of plasmatic glucose and lipid concentrations. Animals with EMS tend to accumulate localized or generalized adipose tissue, are predisposed to rapid weight gain, and have difficulty in mass conversion, and laminitis (Durham et al, 2019; Frank 2009). The ID is usually evidenced by hyperinsulinemia (HI), which presents as a compensatory response to Insulin Resistance (IR). The HI presents as an attempt to internalize glucose by the tissues, which, due to a reduction in the density of insulin receptors, defective signaling pathways or interference in the translocation of GLUT4 transporters do not do so efficiently.

In humans (Boraita 2004) and horses (Bamford et al, 2019), aerobic exercise in addition to caloric restriction is indicated to reduce the signs of metabolic syndrome and improve lipid metabolism. It is also known that low intensity exercise alone does not substantially modify body fat, insulin dynamics or glycemia in horses with EMS. Moderate intensity exercise, however, has been shown to generate an increase in energy consumption that contributes to weight loss and the sensitization of tissues by insulin (Carter et al, 2010; Geor 2010). In this regard, there is a study that demonstrated improved skeletal muscle insulin sensitivity, GLUT4 content and glycogen synthetase activity after a one-month training program in a group of Thoroughbred horses (Bonelli et al, 2017). Studies on GLUT-4 muscle content are contradictory; some authors have not found differences on this regard after exercise (Nout et al, 2003; Pratt et al, 2007), while others describe an increase in GLUT-4 content after a period of exercise training (Lacombe et al, 2003; Stewart et al, 2010). Nevertheless, low and high intensity exercise in horses decrease fat mass after training, although subcutaneous fat thickness does not change, suggesting a decrease in adipose depots other than subcutaneous ones. Swimming in obese horses and horses with EMS has been recommended to avoid the impact of the hoof against the ground under normal training conditions. CPHs are prone to obesity, and it has been suggested that swimming can promote athletic performance and enhance body mass conversion, by promoting the use of energy from adipose tissue. The objective of this study was therefore to evaluate the effect of a swimming training program on the metabolic and endocrine profile (weight, Subcutaneous Fat Thickness - SFT, insulin, cortisol, glucose, triglycerides, cholesterol) of overweight CPHs.

Materials and Methods

Ethical considerations

The procedures carried out on the animals studied were approved by the Animal Experimentation Ethics Committee (CEEA) of the Universidad de Antioquia (Record #122, February 5, 2018).

Study location

The study was carried out in an area located at 6° 9' 0" N and - 75° 22' 1" W, in a "Very Humid Lower Mountainous Forest" (Holdridge 1971), at 2,130 meters above sea level, with an environmental temperature between 12 and 18 °C, and a relative humidity of 96 %. This location corresponded to the horses´ natural environment.

Animals

Six untrained adult CPHs were selected at convenience. Five non-pregnant females and one stallion, with a mean age of 6.6 ± 4.8 (2.5 - 16 years old), a mean weight of 367 ± 41 kg, and a mean body condition of 7/9 according to Henneke scoring system (Henneke 1983) were included. Animals were clinically healthy on physical examination, with a complete and updated health plan (vaccines and deworming) at the time of the measurements. Regarding management conditions, the animals were under complete housing and fed on pangola grass hay (*Digitaria eriantha*; 2.5 kg/day on average), fresh forage (*Pennisetum purpureum*; 30 kg/day on average), commercial balanced feed (2 kg/day on average), mineral formulated salt for horses (100 g/day), and water *ad libitum*. Balanced feed contained a guaranteed composition of protein (20.5%), fat (4%) and fiber (13%). All the animals belonged to the same owner and were housed in the same equestrian center, under the same diet during the study time.

Body fat

Body condition (BCS) was determined in all animals with a classical score scale. In addition, SFT was measured with an ultrasound (ExaGo® veterinary ultrasound scanner, IMV imaging UK Ltd.) using the method validated for CPHs (Zuluaga and Correa-Valencia 2020), in the neck, lumbar, and gluteal regions. These measurements were taken at the onset of the study, at the end of the first training cycle (2 months) and at the end of the second training cycle (4 months). Three measurements were made each time, and the final score was determined by an average of the three, considering a Coefficient of Variation (CV) <0.20 between measurements.

Training protocol

All animals were subjected to a one (1) week adaptation period, after which their training began according to the scheme described in table 1. Exercise intensity was determined by the Maximum Heart Rate (HRmax) obtained in the Field Standardized Exercise Test (f-SET). Swimming training was carried out on three non-consecutive days. All animals underwent discipline-specific exercise training on the two days they did not undergo swimming training and were given an absolute rest day, with no exercise at the end of each week; thus, the swimming training protocol was combined with specific low intensity exercises. The Heart Rate (HR) was assessed using a Bluetooth HR monitor (Ambit 3 vertical®, Suunto Company).

Table 1. Swimming training program for overweight Colombian Paso horses (CPHs).

Cycle	Intensity (HRmax %)	Duration of complete session (minutes)	Number of inmersions	Distance traveled (meters)	Number of swimming days/week
1 (0 - 2 months)	80	10	3	69	3
2 (2 - 4 months)	70	15	4	92	3

HRmax %= percentage of the maximal heart rate reached in the initial field standardized exercise test.

Field Standardized Exercise Test (f-SET)

Athletic performance was evaluated with field standardized exercise test, which were carried out at the onset of the study and at the end of each cycle based on the scheme proposed for CPHs (Arias et al, 2019). Based on the initial f-SET, the HRmax was determined for each horse. Moreover, during in each test, blood samples were obtained at rest and at the end of the maximum intensity step. The f-SET included an initial 15-minute warm-up stage where the horse walked with its rider, maintaining a HR below 140 bpm or at 60% of the HRmax, three exercise sessions of 5 minutes at increasing intensity with a pause of 1 minute among them, in which the horse moved in its gait at a HR between 140 and 160 bpm or between 60 and 70% of the HRmax, 160 and 180 bpm or at 75% of HRmax, and 180 and 200 bpm or between 75 and 85% of HRmax, and finally, a recovery stage in which the horse walked without a rider for 20 minutes.

Lab tests

Blood samples were taken at the beginning of the study and at the end of each cycle during f-SET. At rest and at the end of the maximum intensity step of the f-SET, blood samples were taken to quantify blood concentrations of aspartate aminotransferase (AST), creatine kinase (CK), glucose, insulin, cortisol, triglycerides, and cholesterol. Said samples were collected in a vacuum tube without additive (4 ml of blood).

Statistical analysis

The information obtained was analyzed using descriptive statistics, where all the parameters were shown as median ± standard deviation and inter-quartile range (IQR). An analysis of variance of repeated measures was carried out for non-parametric data (Friedman test), being the three moments of analysis, the beginning of the study, the end of cycle 1 and the end of cycle 2, considering a significance of $p < 0.1$. A correlation analysis was carried out between the SFT and the variables glucose, insulin, cortisol, triglycerides, and cholesterol, using Spearman's Rho coefficient (StataCorp, 2019, College Station, TX, USA).

Results

Effect of swimming training on weight and body condition

Body weight did not show significant changes when the end of each training cycle was compared with the onset of the study, nor between cycles. Body condition on the other hand, changed over time; three animals maintained BCS during the course of the study; Significant differences ($p=0.02$) were observed in two animals that increased their BCS and one which showed a decreased score (table 2).

Table 2. Distribution of body condition in the group of Colombian Paso horses (CPHs) undergoing a four-month swimming training program.

	Initial		*End of cycle 1 (2 months)*		*End of cycle 2 (4 months)*	
BCS	n	%	n	%	n	%
5	0	0,00	1	16,7	1	16,7
6	2	33,3	2	33,3	2	33,3
7	2	33,3	0	0,00	0	0,00
8	2	33,3	3	50,0	3	50,0

BCS= Body condition score by Henneke.

Effect of swimming training on body fat

No significant differences were observed in SFT changes between the neck and lumbar regions during the course of both training cycles. On the other hand, SFT decreased significantly (p=0.05) in the gluteal region and proportionally in all animals when compared with the initial measurement and the end of each training cycle (figure 1).

Figure 1. Gluteal Subcutaneous Fat Thickness (SFT).

Effect of swimming training on triglycerides and cholesterol

Blood triglyceride concentration was different at rest and post-exercise in all f-SET (initial, end of cycle 1 and end of cycle 2), while blood cholesterol concentration was different at rest and post-exercise in the initial and final f-SET of cycle 1, but not at the end of cycle 2. Furthermore, blood cholesterol concentration was significantly different between tests (table 3).

Table 3. Median, standard deviation, Inter-quartile range of blood triglyceride and cholesterol concentration in a group of Colombian Paso horses (CPHs) subjected to a four-month swimming training program.

Parameter	*Timing of f- SET*	*Initial* median ± SD (IQR)	*End of cycle 1 (2 months)* median ± SD (IQR)	*End of cycle 2 (4 months)* median ± SD (IQR)	Reference range
Tryglicerides (mg/dL)	Rest	31.5 ± 26.5[a] (26.1 - 58.4)	37.8 ± 18.5[b] (26.5 - 54.5)	55.8 ± 6.9[c] (52.3 - 2.7)	11–52
	Post-exercise	57 ± 12.3[a] (42.6 – 59.7)	55.9 ± 14.2 [b] (49.3 – 64.2)	76.3 ± 18.3 (66.5-99.6)	
Cholesterol (mg/dL)	Rest	120.4 ± 24[a*] (101.9-134.3)	113.2 ± 31.8[b*] (70.1-1245)	69.7 ± 44[*] (46.8-127.7)	51–109

	Post-exercise	91.3 ± 48.9[a*] (58.3-131.2)	124.3 ± 59.5[b*] (85.3-156)	71.6 ± 37[*] (42.4-10.5)

f-SET= Field Standardized Exercise Test; IQR= Interquartile Range; SD= Standard Deviation; p≤0.1 according to the Friedman test*, differences between cycles; a. differences between values at rest and post-exercise in the f-SET at the end of cycle 1; b. differences between values at rest and post-exercise in the f-SET at the end of cycle 2). Reference range for triglycerides and cholesterol taken from (Southwood 2013).

Effect of swimming training on hormones and glycemia

Even though glycemia remained within the physiological range for the species 75 –115 mg / dL (Kaneko et al, 2008) during all the f-SET, insulin behavior was variable (figure 2). At the onset of the study, prior to training, post-exercise insulin was higher than resting insulin. In contrast, once training cycles 1 and 2 ended, said behavior was reversed, with insulin levels being higher at rest compared to post-exercise insulinemia. As noted in table 3, insulin concentration exceeded the reference range, especially at rest.

Cortisol concentrations did not exceed the reference range at rest, however, at post-exercise it was higher than the resting value and higher than the reference range in all exercise tests (table 4). Table 5 shows the neck, lumbar and gluteal SFT obtained from every horse under the swimming training.

Figure 2. Blood insulin before and after the Field Standardized Exercise Test (f-SET).

Table 4. Median, standard deviation, and Inter-quartile range of blood cortisol, insulin, and glucose concentration in a group of Colombian Paso horses (CPHs) subjected to a four-month swimming training program.

Parameter	Timing of Stress Test	*Initial* median ± SD (IQR)	*End of cycle 1 (2 months)* median ± SD (IQR)	*End of cycle 2 (4 months)* median ± SD (IQR)	Reference range
Cortisol (mg/dL)	Rest	8.4 ± 3.3	9.4 ± 3.1[a]	11.7 ± 5.6[b]	3.0 - 13

		(7.0 - 13.1)	(8.2 - 9.9)	(8.9 - 16.9)	
	Post-exercise	12.3 ± 7.3 (8.0 - 16.5)	15.8 ± 5.9[a] (14.0 - 19.7)	14.7 ± 10.3[b] (13.3-24.4)	
Insulin (µUI/mL)	Rest	43.5 ± 22.7[a] (6.5 - 46.0)	27 ± 49.1 (10.3 - 27.0)	57 ± 38.3 (10.1 - 80)	5,03-60,84
	Post-exercise	47.1 ± 23.1[a] (22.9 - 64.3)	20 ± 10.3 (18.1 - 26.8)	21.3 ± 20.1 (14.4 - 51.3)	
Glucose (mg/dL)	Rest	90.5 ± 12.9 (85 - 99)	104.5 ± 9.1 (96 - 112)	100.5 ± 9.3 (98 - 106)	72–114
	Post-exercise	114 ± 22.4 (101 - 124)	120 ± 13.7 (111 - 133)	116 ± 7.8 (112 - 127)	

IQR= Interquartile range; SD= Standard deviation; p<0.1 according to the Friedman test*, differences between cycles; a. differences between values at rest value and post-exercise in the f-SET at the end of cycle 1; b. differences between values at rest value and post-exercise in the final f-SET of cycle 2. Reference range for cortisol, insulin and glucose was taken from (Zuluaga and Martínez 2017), (Warnken et al, 2016) and (Southwood 2013), respectively.

Table 5. Neck, lumbar and gluteal Subcutaneous Fat Thickness (SFT) obtained from every horse submitted to the swimming training.

	Neck SFT (cm)			Lumbar SFT (cm)			gluteal SFT (cm)		
Animal	*Initial*	*End of cycle 1*	*End of cycle 2*	*Initial*	*End of cycle 1*	*End of cycle 2*	*Initial*	*End of cycle 1*	*End of cycle 2*
Horse 1	1,2	7,4	5,8	8,3	4,7	3,1	9,5	4,5	5,8
Horse 2	6,4	3,9	3,4	7,4	6,3	3,4	4,5	2,7	0,0
Horse 3	1,2	2,4	3,0	3,8	3,4	5,0	4,0	5,5	6,8
Horse 4	7,0	0,0	0,0	7,0	4,8	4,7	5,9	3,4	3,2
Horse 5	6,7	4,5	0,0	6,0	6,1	5,4	4,0	4,1	0,0
Horse 6	6,2	6,7	0,0	4,5	4,3	4,3	5,9	5,6	3,7

SFT= Subcutaneous Fat Thickness.

Effect of swimming on muscle enzymes

The concentration of CK and AST was quantified both at rest and at the end of the exercise tests, at the three times previously indicated. [CK] at rest in no case exceeded the reference range for the species 116 - 290 U / L (Hodgson et al, 2014), however, after exercise both at the end of cycle 1 and at the end of cycle 2 the median showed an increase with respect to rest and above the reference value (336 and 332 U / L respectively). The AST, on the other hand, was higher post-exercise in all tests, although only at the end of cycle 1 did it exceed the reference range 226–366 U / L (Kaneko et al, 2008) with a median of 420 U / L.

Discussion

Effect of swimming training on weight and body condition

Fatty tissue does not participate in athletic performance as skeletal muscle does, therefore, the percentage of muscle in relation to body weight is of greater interest than weight itself. The findings of the present study suggest that, during the application of a training protocol of decreasing intensity in a pool, horses can experience mass conversion, that is, reduction of adipose tissue and changes in body condition, without significantly altering body weight. Some studies found significant changes in the decrease in weight in horses subjected to low intensity exercise and dietary restriction, as well as a decrease in body condition with an increase of muscle mass (Moore et al, 2019; Bamford et al, 2019). Although muscle mass was not evaluated in the present study, the most feasible explanation for the reduction in adipose tissue without changes in weight is that it was replaced by muscle mass.

Effect of swimming training on body fat

Morphometric estimates were not used due to the lack of sensitivity of the indicators based on neck circumference in CPHs (Zuluaga and Correa-Valencia 2020). However, it was found that overall SFT decreased systematically during training, especially adipose tissue of the gluteal region ($p=0.05$). These findings suggest that this type of training can be effective in the management of obesity in CPHs, allowing the maintenance of athletic performance without the impact on the hoof.

Researchers showed that both low and high intensity exercise do not alter SFT (Carter et al, 2010). However, it should be noted that the pattern of body fat deposition and the type of exercise applied to a particular breed do not have comparable behaviors. For example, lumbar SFT in untrained horses and then subjected to treadmill training was less than 9.6 mm ± 0.15, while on the other hand, in the CPHs of the present study, initial SFT was 6.5 mm ± 1.2 and 4.5 mm ± 0.9 after twelve weeks of swimming training (Carter et al, 2010).

Although neck SFT did not show any trend during the study, it was found that those animals that at the beginning of the study registered a thickness between 6 and 7 mm, at the end of cycle 2, thickness had decreased between 3 and 6 mm (n=4); moreover, the animals that registered neck SFT between 1 and 1.5 mm increased its thickness at the end of cycle 2 (n=2) (Table 5). Given this, it could be hypothesized that the body fat redistribution phenomenon may exist during a swimming training protocol such as the one designed for this study. In addition, it is also known that according to the muscle groups that work during exercise, it could also be the mobilization of body fat; in the case of swimming in horses, it is recognized that the gluteal muscles work directly (Boffi 2008). An *in vivo* study, showed that rats with metabolic syndrome subjected to exercise generated a remodeling of adipose tissue due to an adaptive muscle change, and it found through thermogenesis that the gastrocnemius muscle increased its function as insulin regulator, observing a decrease around adipocytes and an increase in neovascularization (Barbosa et al, 2018).

It should be clarified that the values of 0 mm in the SFT refer to inestimable values by the equipment, given that it is biologically impossible for subcutaneous fat to be absent.

Effect of swimming training on insulin, cortisol and glycemia

Although the animals were not previously diagnosed with IR, hyperinsulinemia was evidenced at rest as previously reported in animals with EMS. The adaptations produced by exercise in skeletal muscle such as neovascularization may be involved in increasing glucose uptake, so it is logical to assume that as the muscle improves the use of energy substrates, the demand for insulin decreases.

The beneficial effect of moderate intensity training on insulin levels and glucose mobilization has been demonstrated, which has been attributed to the activity of glucose-regulating hormones, and to the release of nitric oxide by the vascular endothelium, favoring glucose uptake (Bonelli et al, 2017; Geor et al, 2010; Turner et al, 2011). The acute effect of exercise on glucose could be observed in the animals subjected to swimming training at the end of exercise in the initial phase, at the end of cycle 1 and the end of cycle 2. This behavior is expected, given that it is a classic physiological response triggered by hyperglycemic hormones during exercise (Hyyppä 2005).

In the present study, cortisol levels increased during post-exercise periods compared with those observed at rest. As has been described by several authors, high intensity exercise is a physical stress that triggers the secretion of cortisol, increasing lipolysis to meet the energy demands of active skeletal muscle, contributing to a decrease in adipose tissue (Hyyppä 2005; Kędzierski and Cywińska 2014). This partially explains the loss of adipose tissue observed in horses in this study.

The acute effect of swimming on plasmatic insulin concentration levels was observed at the end of cycles 1 and 2, suggesting that this hormonal adaptation requires a training period of at least two months.

Effect of swimming training on triglycerides and cholesterol

As described in other study, moderate and high intensity exercise stimulates the mobilization of triglycerides, which is reflected in the increase in their blood concentration (Carter et al, 2010). The fatty acids used in muscle metabolism come from adipose tissue, circulating lipoproteins or triglycerides stored in the muscle cell. Sympathetic-adrenal activation and decreased insulin concentrations are the main stimuli for lipolysis during exercise.

Endurance training is associated with an increase in beta-adrenergic sensitivity in adipose tissue, which causes greater consumption of fatty acids as an energy source, and an improvement in the capacity for oxidation of fatty tissue and mitochondrial activity (Connolly et al, 2016). The results found in this study are similar to other research, where authors observed that the values of stratified fatty acids and triglycerides in horses increase significantly at the end of exercise (Assenza et al, 2016); subjects evaluated in this study revealed an increase in triglycerides at the end of each of the f-SET, although no changes were recorded over time. In contrast, significant differences were observed in cholesterol levels between the different evaluation periods, suggesting a possible adaptation of swimming training in CPHs is a decrease in blood cholesterol.

Effect of swimming training on muscle activity

Aerobic exercise combined with caloric restriction is indicated to reduce the signs of metabolic syndrome and improve the expression of GLUT4 transporters in the plasma membrane of skeletal muscle, as well as to improve lipid metabolism and increase mitochondrial oxidation both in humans (Boraita 2004) and in horses (Bamford et al, 2019); moderate intensity exercise, therefore, has shown to generate an increase in energy consumption that contributes to weight loss and insulin sensitization by the tissues (Flores-Opazo et al, 2020). In this regard, the findings of this study indicate that the training protocol used, classified as moderate, coincide with what has been previously reported.

Other authors reported in a similar study, that a one-month pool training program, improved insulin sensitivity, concluding that moderate intensity exercise promotes glucose mobilization and modifies hormonal response, glucose regulators, increasing insulin sensitivity, GLUT-4 content, and glycogen synthetase activity in skeletal muscle. Adipose tissue and skeletal muscle are the main glucose absorption tissues, being highly dependent on insulin for the expression of

GLUT4, as well as the liver which responds to insulin secretion by increasing glucose utilization (Durham et al, 2019; Frank 2009; Frank 2011).

Likewise, the earliest and most common muscular adaptation to resistance training is the increase in enzymatic activity of aerobic metabolism, such as the enzymes in the Krebs cycle, the mitochondrial respiratory chain and β oxidation, which is associated with increased mitochondrial and capillary density (Barbosa et al, 2018). Increased post-exercise glucose and triglyceride levels observed in this study suggest that these energy substrates are available for use in muscle oxidative metabolism. No abnormalities in CK and AST blood concentrations were found indicating no muscle damage.

Conclusion

A moderate aerobic exercise swimming training program proved effective in reducing body fat, becoming a therapeutic option for overweight and obese horses. Said protocol requires a minimum of 2 months to observe changes in plasmatic insulin concentration levels and could be included in the treatment of EMS to improve insulin sensitivity. However, further studies with larger and controlled sample are recommended in order to obtain more conclusive results in CPHs due to their tendency to suffer from obesity.

References

Arias, M.P., Maya, J.S., Arango, L., 2019. Efectos de dos protocolos de entrenamiento sobre el lactato sanguíneo en caballos de paso fino. Revista de la Facultad de Medicina Veterinaria y Zootecnia 66(3):219-230. http://www.doi.org/10.11606/issn.1678-4456.bjvras.2020.171082

Assenza, A., Arfuso, F., Zanghì, E., Fazio, F., Bruschetta, D., Piccione, G., 2016. Lipid and Lipoprotein Profiles Modification in Athletic Horses After Repeated Jumping Events. Journal of Equine Veterinary Science 43:28-31. http://www.doi.org/10.1016/j.jevs.2016.05.001

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. http://www.doi.org/10.1111/jvim.15374

Barbosa, M.A., Guerra-Sá, R., De Castro, U.G.M., de Lima, W.G., Dos Santos, R.A.S., Campagnole-Santos, M.J., Carvalho, A., 2018. Physical training improves thermogenesis and insulin pathway and induces remodeling in white and brown adipose tissues. Journal of Physiology Biochemistry 74(3):441-454. http://www.doi.org/10.1007/s13105-018-0637-x

Boffi, F.M., 2008. Entrenamiento y adaptación muscular: sustratos y vías metabólicas para la producción de energía. Revista Brasilera de Zootecnia 37(spe):197-201. http://www.doi.org/10.1590/S1516-35982008001300022

Bonelli, F., Sgorbini, M., Meucci, V., Sighieri, C., Baragli, P., 2017. How swimming affects plasma insulin and glucose concentration in Thoroughbreds: A pilot study. Veterinary Journal 226:1-3. http://www.doi.org/10.1016/j.tvjl.2017.06.006

Boraita, A., 2004. Plasma lipid profile is improved by participation in sports, but at what intensivity?. Revista Española de Cardiología 57(6):495-498. http://www.doi.org/ 10.1016/S1885-5857(06)60624-5

Carter, R.A., McCutcheon, L.J., Valle, E., Meilahn, E.N., Geor, R.J., 2010. Effects of exercise training on adiposity, insulin sensitivity, and plasma hormone and lipid concentrations in overweight or obese, insulin-resistant horses. American Journal of Veterinary Research 71(3):314-21. http://www.doi.org/10.2460/ajvr.71.3.314

Connolly, L.J., Nordsborg, N.B., Nyberg, M., Weihe, P., Krustrup, P., Mohr, M., 2016. Low-volume high-intensity swim training is superior to high-volume low-intensity training in relation to insulin sensitivity and glucose control in inactive middle-aged women. European Journal of Applied Physiology 116(10):1889-97. http://www.doi.org/10.1007/s00421-016-3441-8

Durham, A.E., Frank, N., McGowan, C.M., Menzies-Gow, N.J., Roelfsema, E., Vervuert, I., Feige, K., Fey, Kerstin., 2019. ECEIM consensus statement on equine metabolic syndrome. Journal of Veterinary Internal Medicine 33(2):335-49. http://www.doi.org/10.1111/jvim.15423

Espíndola-Antunes, D., Kater, C.E., 2007. Adipose tissue expression of 11beta-Hydroxysteroid dehydrogenase type 1 in cushing's syndrome and in obesity. Arquivo Brasileiro de Endocrinologia y Metabolismo 51(8):1397-403. http://www.doi.org/10.1590/S0004-27302007000800027

Flores-Opazo, M., McGee, S.L., Hargreaves, M., 2020. Exercise and GLUT4. Exercise and Sport Sciences Reviews 48(3):110-118. http://www.doi.org/10.1249/JES.0000000000000224

Frank, N., 2009. Equine Metabolic Syndrome. Journal of Equine Veterinary Science 29(5):259-67. http://www.doi.org/10.1016/j.jevs.2009.04.183

Frank, N., 2011. Equine Metabolic Syndrome. Veterinary Clinics of North America Equine Practice 27(1):73-92. http://www.doi.org/10.1016/j.cveq.2010.12.004

Geor, R.J., Mccutcheon, L.J., Hinchcliff, K.W., Sams, R.A., 2010. Training-induced alterations in glucose metabolism during moderate-intensity exercise. Equine Veterinary Journal 34(S34):22-8. http://www.doi.org/10.1111/j.2042-3306.2002.tb05386.x

Geor, R.J., 2010. Nutrition and Exercise in the Management of Horses and Ponies at High Risk for Laminitis. Journal of Equine Veterinary Science 30(9):463-70. http://www.doi.org/10.1016/j.jevs.2010.07.011

Henneke, D.R., Potter, 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):371-2. http://www.doi.org/10.1111/j.2042-3306.1983.tb01826.x

Hodgson, D.R., McKeever, K.H., McGowan, C.M. (eds.), 2014. The athletic horse: principles and practice of equine sports medicine. 2nd ed. Saunders/Elsevier, St. Louis, USA.

Holdridge, L.R., 1971. Forest environments in tropical life zones: a pilot study. 1st ed. Pergamon Press, New York, USA.

Hyyppä, S., 2005. Endocrinal responses in exercising horses. Livestock Production Science 92(2):113-21. http://www.doi.org/10.1016/j.livprodsci.2004.11.014

Kaneko, J.J., Harvey, J.W., Bruss, M. (eds.), 2008. Clinical biochemistry of domestic animals. 6th ed. Academic Press/Elsevier, Boston, USA.

Kędzierski, W., Cywińska, A., 2014. The Effect of Different Physical Exercise on Plasma Leptin, Cortisol, and Some Energetic Parameters Concentrations in Purebred Arabian Horses. Journal of Equine Veterinary Science 34(9):1059-63. http://www.doi.org/10.1016/j.jevs.2014.06.005

Lacombe, V.A., Hinchcliff, K.W., Devor, S.T., 2003. Effects of exercise and glucose administration on content of insulin-sensitive glucose transporter in equine skeletal muscle. American Journal of Veterinary Research 64(12):1500-6. http://www.doi.org/10.2460/ajvr.2003.64.1500

Moore, J.L., Siciliano, P.D., Pratt-Phillips, S.E., 2019. Effects of Diet Versus Exercise on Morphometric Measurements, Blood Hormone Concentrations, and Oral Sugar Test Response in Obese Horses. Journal of Equine Veterinary Science 78:38-45. http://www.doi.org/10.1016/j.jevs.2019.03.214

Nout, Y.S., Hinchcliff, K.W., Jose-Cunilleras, E., Dearth, L.R., Sivko, G.S., DeWille, J.W., 2003. Effect of moderate exercise immediately followed by induced hyperglycemia on gene expression and content of the glucose transporter-4 protein in skeletal muscles of horses. American Journal of Veterinary Research 64(11):1401-8. http://www.doi.org/10.2460/ajvr.2003.64.1401

Pratt, S.E., Geor, R.J., Spriet, L.L., McCutcheon, L.J., 2007. Time course of insulin sensitivity and skeletal muscle glycogen synthase activity after a single bout of exercise in horses. Journal of Applied Physiology 103(3):1063-9. http://www.doi.org/10.1152/japplphysiol.01349.2006

Reynolds, A., Keen, J.A., Fordham, T., Morgan, R.A., 2019. Adipose tissue dysfunction in obese horses with equine metabolic syndrome. Equine Veterinary Journal 51(6):760-766. http://www.doi.org/10.1111/evj.13097

Stewart-Hunt, L., Pratt-Phillips, S., McCutcheon, L.J., Geor, R.J., 2010. Dietary energy source and physical conditioning affect insulin sensitivity and skeletal muscle glucose metabolism in horses. Equine Veterinary Journal Supplement 38:355-60. http://www.doi.org/10.1111/j.2042-3306.2010.00255.x

Southwood, L.L., 2013. Practical guide to equine colic. 1st ed. Wiley-Blackwell, Iowa, USA.

Turner, S.P., Hess, T.M., Treiber, K., Mello, E.B., Souza, B.G., Almeida, F.Q., 2011. Comparison of Insulin Sensitivity of Horses Adapted to Different Exercise Intensities. Journal of Equine Veterinary Science 31(11):645-9. http://www.doi.org/10.1016/j.jevs.2011.05.006

Warnken, T., Huber, K., Feige, K., 2016. Comparison of three different methods for the quantification of equine insulin. BMC Veterinary Research 12(1):196. http://www.doi.org/10.1186/s12917-016-0828-z

Zuluaga, A.M., Martínez, J.R., 2017. Serum cortisol concentration in the Colombian Creole Horse. Revista Colombiana de Ciencias Pecuarias 30(3):231-238. https://doi.org/10.17533/udea.rccp.v30n3a06

Zuluaga, A.M., Correa-Valencia, N.M., 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. http://www.doi.org/10.11606/issn.1678-4456.bjvras.2020.171082

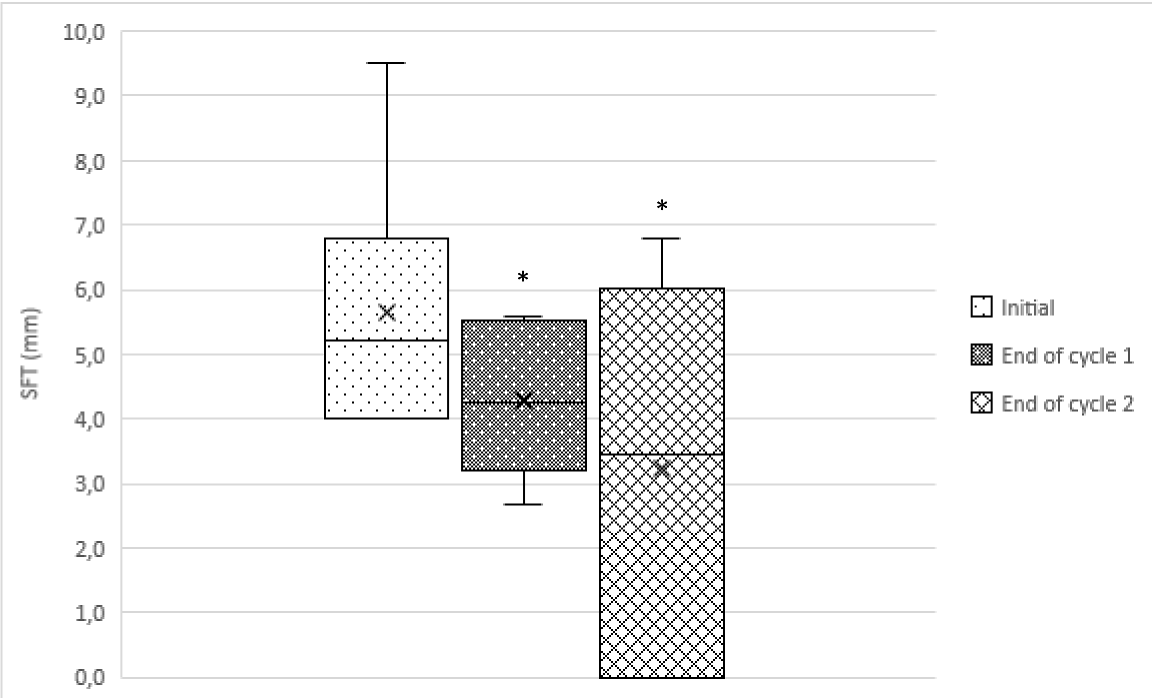

Figure 1. **Gluteal Subcutaneous Fat Thickness (SFT).** Distribution of the thickness of adipose tissue of the gluteal region in a group of Colombian Paso horses (CPHs) at the onset of the study and at the end of cycle 1 and 2, subjected to a swimming training program. * P value=0.05 (Friedman test).

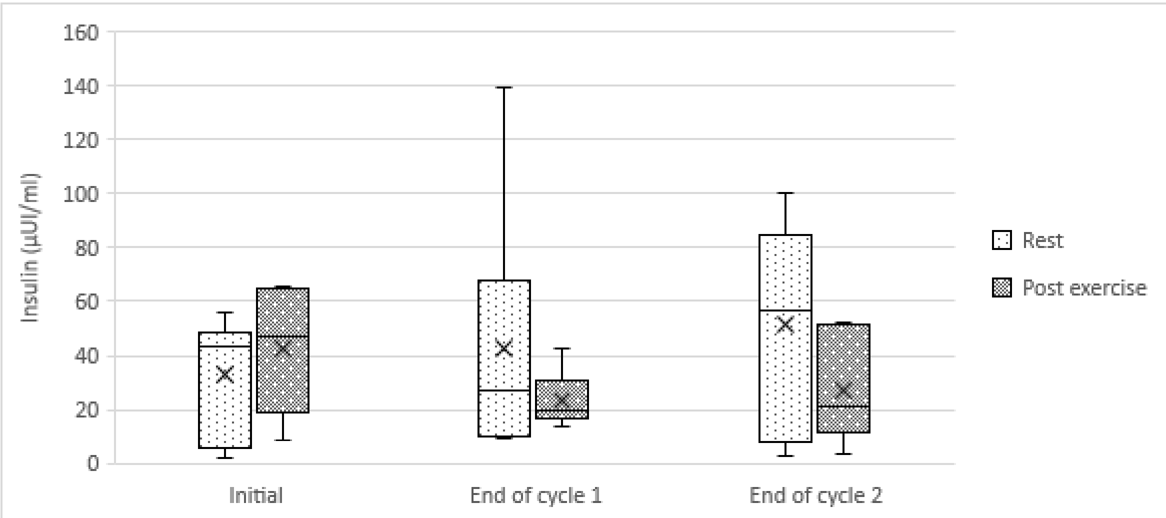

Figure 2. **Blood insulin before and after the Field Standardized Exercise Test (f-SET).** Distribution of blood insulin concentration at rest and after the field standardized exercise test at the onset of the study and at the end of cycles 1 and 2, in Colombian Paso horses (CPHs) subjected to swimming training program.

Capítulo 4. Adaptaciones cardiovasculares, metabólicas y hematológicas, electrolíticas y ácido-base en caballos de paso, entrenados bajo un protocolo en tierra combinado con natación.

En este capítulo se describen las respuestas y adaptaciones logradas por los animales del estudio una vez finalizó el programa de entrenamiento cruzado en piscina. Estos resultados responden a los objetivos específicos 1,2 y 3.

Adaptaciones cardiovasculares, metabólicas y hematológicas, electrolíticas y ácido-base en caballos de paso colombiano, entrenados bajo un protocolo de entrenamiento en tierra combinado con natación.

Angélica M Zuluaga Cabrera, Nathalia MDP Correa-Valencia, María P Arias Gutiérrez

Resumen

Antecedentes: Los efectos del entrenamiento en tierra combinado con natación no se han registrado hasta ahora en el caballo de paso colombiano (CPC). Objetivo: Describir las adaptaciones cardiovasculares, metabólicas y hematológicas, electrolíticas y ácido-base en CPC, entrenados bajo un protocolo de entrenamiento en tierra combinado con natación. Métodos: Seis CPC, adultos, desentrenados, fueron sometidos a un protocolo de entrenamiento en tierra combinado con natación a una intensidad decreciente durante un periodo de 4 meses. El desempeño atlético fue evaluado a través de pruebas de esfuerzo estandarizas en campo. Resultados: La frecuencia cardiaca máxima disminuyó en el transcurso del entrenamiento. El aclaramiento del lactato sanguíneo (LS) fue mayor cuando se comparó el inicio del programa con el final del ciclo 2. Por su parte, no se encontraron cambios en las variables hematológicas o bioquímicas. Conclusión: El protocolo de entrenamiento diseñado produjo adaptaciones cardiovasculares y metabólicas que mejoraron la capacidad aerobia de los animales, sin producir alteraciones hematológicas y bioquímicas derivadas del ejercicio.

Palabras clave: equino, frecuencímetro, umbral anaerobio, lactato

Introducción

La combinación de ejercicios propios de la disciplina ecuestre con una actividad física paralela como la natación se realiza con frecuencia en caballos de deporte, con el objetivo de diversificar el trabajo muscular, mejorar la capacidad cardiovascular y respiratoria; además, la natación ayuda a mantener una actitud positiva hacia el ejercicio, al alternar la rutina de entrenamiento [1].

Se sabe que el entrenamiento de natación ayuda a mejorar la capacidad cardio-respiratoria y el metabolismo aerobio, como principales adaptaciones fisiológicas en humanos [2]. En equinos, algunas respuestas fisiológicas agudas a la natación de tipo respiratorio, cardiovascular y metabólico han sido estudiadas [3–5]. Sin embargo, las adaptaciones fisiológicas crónicas como consecuencia del entrenamiento en piscina solo o en combinación con otro tipo de ejercicio no han sido descritas en caballos de paso colombiano (CPC) a la fecha.

Por lo tanto, el objetivo del presente estudio fue describir las adaptaciones cardiovasculares (frecuencia cardiaca en reposo y la frecuencia cardiaca máxima), metabólicas (lactato sanguíneo-LS, creatinina, nitrógeno ureico sanguíneo-BUN, gamma-glutamil-transaminasa-GGT, fosfatasa alcalina-FA), hematológicas (hemoleucograma), electrolíticas y ácido-base en CPC, entrenados bajo un protocolo de entrenamiento en tierra combinado con natación.

Materiales y métodos

Consideraciones éticas:

Los procedimientos realizados en los animales de estudio fueron aprobados por el Comité de Ética para la Experimentación con Animales (CEEA) de la Universidad de Antioquia (acta # 122, 5 de febrero de 2018).

Instalaciones:

El estudio fue realizado en instalaciones de una entidad privada ubicada en el municipio de Rionegro (Antioquia), a una altitud de 2.080 msnm y humedad relativa del 70% (IDEAM, 2018). La piscina utilizada fue diseñada para equinos, sus medidas son: 3 metros de radio interno, 2.40 metros de profundidad, 23.3 metros de recorrido máximo según su circunferencia más amplia (figura 1), y 5 metros de pasillo de entrada o salida en pendiente (figura 2). Adicionalmente, cuenta con 2 motores de alto caudal para llevar a cabo procesos de limpieza y acondicionamiento del agua. Cuenta con una isla central con el fin de permitir la conducción a dos cuerdas de los animales.

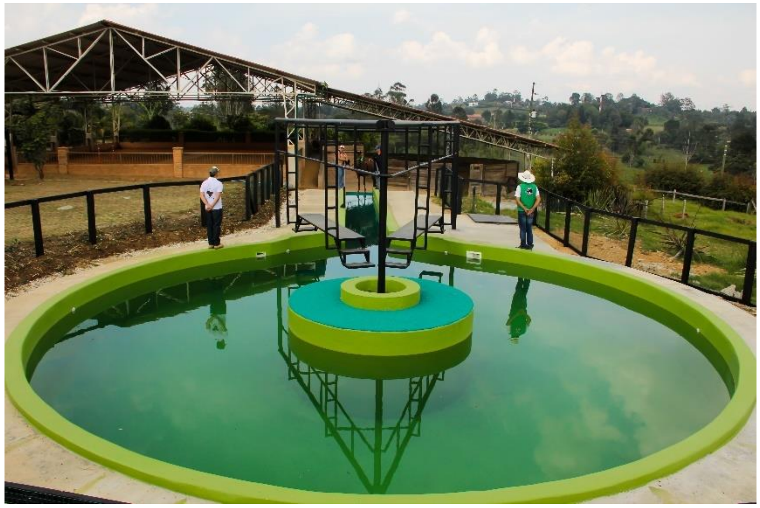

Figura 1. Aspecto general de la piscina para equinos

Figura 2. Ingreso a la piscina para equinos

Los alojamientos donde fueron estabulados los animales tienen las dimensiones: 3.80 m de largo, 2.40 m de ancho y 1.30 m de altura. Los comederos midieron 2.40 m de largo, 65 cm de alto y 56 cm de ancho (figura 3). Todo el alojamiento, incluyendo el comedero, se encuentra construido en cemento y ladrillo. El bebedero es automático.

Figura 3. Alojamientos de ejemplares objeto de estudio

Evaluación clínica:

Cada ejemplar fue sometido a examen clínico previo a su ingreso al estudio, y se descartó la presentación de patologías contraindicadas para la natación, como lesiones lumbo-sacras y artrosis.

Animales

Seis (6) CPC fueron elegidos a conveniencia. Cinco (5) hembras no-gestantes y un (1) caballo entero, con una edad promedio de 6.6 ± 4.8 años (2.5 - 16 años), peso promedio de 367 ± 41 kg, y condición corporal promedio de 7/9 según la escala de Henneke [6] fueron incluidos. Los animales estuvieron completamente estabulados, alimentados con heno Pangola (*Digitaria eriantha*; 2.5 kg/d en promedio), forraje verde (*Pennisetum purpureum*; 30 kg/d en promedio), alimento balanceado comercial (2 kg/d en promedio), sal mineral formulada para caballos (100 g/d), y agua *ad libitum*. Los animales pertenecían al mismo propietario y se alojaron en el mismo centro ecuestre.

Prueba de esfuerzo

El desempeño atlético fue evaluado a través de pruebas de esfuerzo en campo. Las pruebas de esfuerzo en campo se realizaron al iniciar el estudio y luego, al finalizar cada ciclo (cada 2 meses); el esquema de las pruebas de esfuerzo fue tomado de Arias *et al*. (2019) (figura 4) [7]. A partir de la prueba de esfuerzo inicial se determinó la FCmax (frecuencia cardiaca que se mantuvo en su estado estable durante el esfuerzo máximo) de cada caballo. Por otro lado, en cada prueba de esfuerzo se obtuvieron muestras de sangre en reposo y al finalizar el escalón de máxima intensidad.

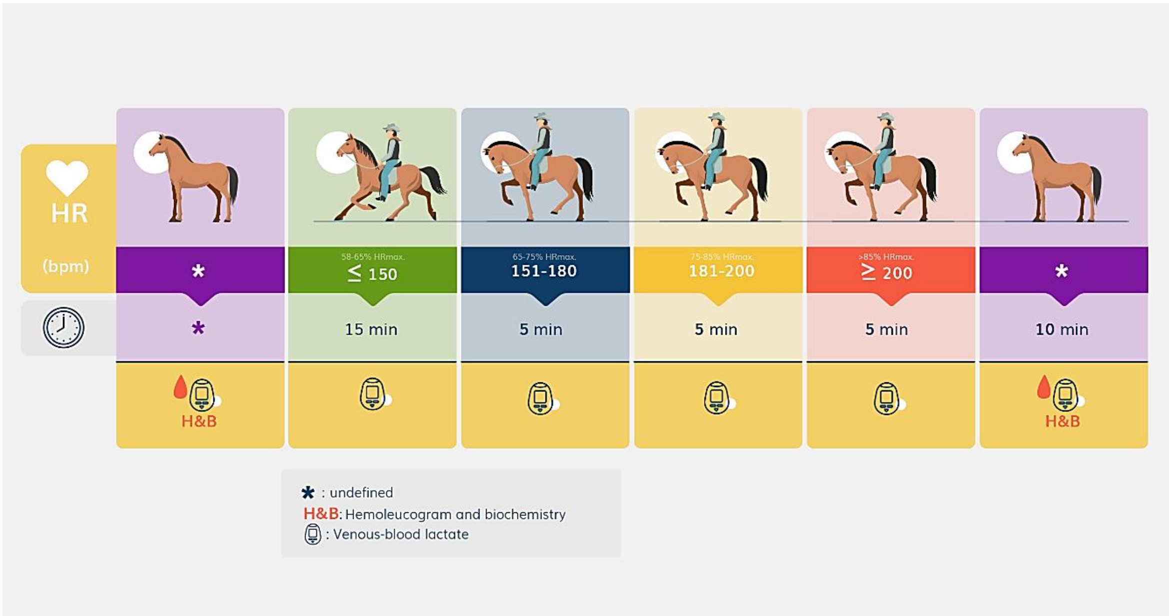

Figura 4. Ilustración de la prueba de esfuerzo en campo para caballos de paso colombiano (CPC), frecuencia cardiaca, duración y muestras sanguíneas tomadas.

Protocolo de entrenamiento:

Todos los animales fueron sometidos a una (1) semana de adaptación. Una vez transcurrida esta semana, los animales iniciaron su entrenamiento de acuerdo con el esquema que se encuentra en la tabla 1 y figura 4. La intensidad del ejercicio fue determinada a partir de la FCmax obtenida en la prueba de esfuerzo inicial. Todos los animales experimentaron entrenamiento de ejercicios específicos de la disciplina durante los 3 días alternos a la natación y, además, se les otorgó un día de descanso absoluto al finalizar cada semana. La frecuencia cardíaca fue medida utilizando un frecuencímetro marca *Suunto Ambit3 Vertical*®.

Tabla 1. Protocolo de entrenamiento de natación para caballos de paso colombiano (CPC) con sobrepeso.

Ciclo	Intensidad (% de la FC máx.)	Duración total de la sesión (minutos)	Número de inmersiones	Distancia recorrida en metros	Número de días de natación a la semana
1 (0 a 2 meses)	80	10	3	69	3
2 (mes 4)	70	15	4	92	3

Pruebas de laboratorio:

Se tomaron muestras de sangre en cada prueba de esfuerzo (inicial, final del ciclo 1 y final del ciclo 2) durante el reposo y la fase de máxima intensidad. Se tomaron muestras en tubo de vacío con aditivos: EDTA (2 ml de sangre), además de tubo sin aditivo (4 ml de sangre) y jeringas con heparina de litio (1.5 ml de sangre) (figura 4).

Fueron realizados hemoleucograma, proteínas diferenciadas, creatinina, BUN, GGT, FA. La concentración de albúmina y proteínas totales fueron utilizadas para calcular el valor de ATOT y el cambio en el volumen plasmático pos-ejercicio. Adicionalmente se utilizó un analizador portátil de iones y gases sanguíneos *EPOC*® blood analysis system (Erlangen, Alemania) para obtener la concentración sanguínea de sodio, potasio, cloro, calcio ionizado, lactato, bicarbonato y glucosa, además del valor de pH y bases efectivas (BE). La concentración de los iones se utilizó para calcular la diferencia de iones fuertes (SID). El lactato sanguíneo fue tomado en reposo, cada etapa de la prueba de esfuerzo y en la recuperación (figura 4), utilizando un medidor portátil Nova

Plus® (Biomedical, Waltham, MA, United States). Con base en la medición del lactato sanguíneo en cada escalón y cada ciclo se calculó el umbral aerobio (FC2) y el umbral anaerobio (FC4), utilizando la función de regresión exponencial de Microsoft Excel®.

El análisis de iones en sangre venosa también fue realizado en reposo y posterior a la última sesión del mes 1 de entrenamiento en piscina, luego en reposo y posterior a la última sesión del mes 5 de entrenamiento en piscina.

Análisis estadístico

Se realizó un análisis de varianza de medidas repetidas para datos no paramétricos (prueba de Friedman; $p<0,1$), siendo los momentos de análisis, el inicio del estudio, el final del ciclo 1 y el final del ciclo 2. Para todos los parámetros de estudio se reporta mediana ± desviación estándar y rango inter-cuartil (RIC).

Resultados

Adaptación cardiovascular

La frecuencia cardiaca en reposo experimentó cambios entre el mes 1 y mes 3; sin embargo, al finalizar el mes 4 dicho parámetro regresó al valor registrado durante el mes 1 (figura 4). La frecuencia cardiaca máxima obtenida en las sesiones de entrenamiento en piscina durante los 4 meses disminuyó linealmente (figura 5).

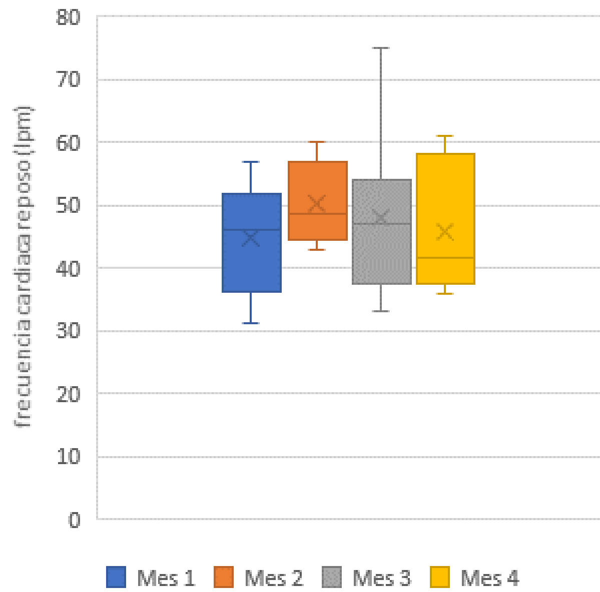

Figura 4. Distribución de la frecuencia cardiaca en reposo durante la última sesión de natación al finalizar cada mes de entrenamiento.

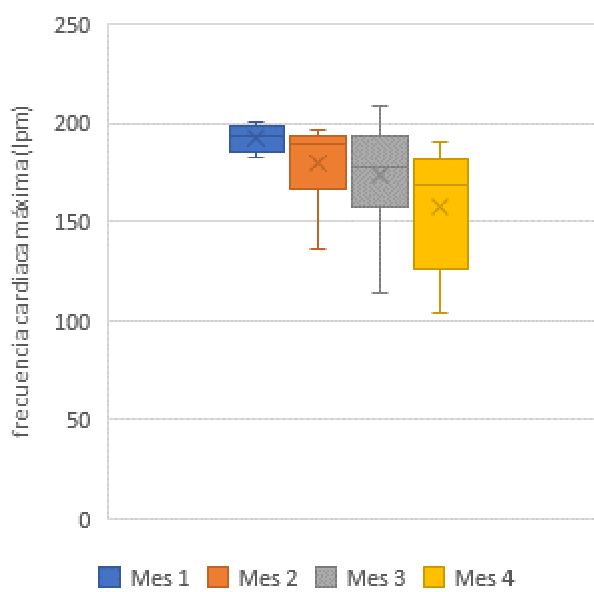

Figura 5. Distribución de la frecuencia cardiaca máxima obtenida en la última sesión de natación y al final de cada mes de entrenamiento.

Parámetros hematológicos

Ninguno de los valores relacionados con el conteo de eritrocitos, índices eritrocitarios, conteo de leucocitos (total y diferenciado), conteo de plaquetas, y proteínas mostraron diferencias estadísticamente significativas entre ciclos. Un efecto reconocible en los resultados fue la hemoconcentración que sucedió por deshidratación durante el ejercicio (tabla 2).

Cambios electrolíticos y ácido-base

La concentración de LS fue significativamente diferente ($p<0,1$) en la etapa de máxima intensidad en todas las pruebas de esfuerzo. El valor en el reposo fue bajo (alrededor de 1 mmol/L) en el inicio del estudio, al final del ciclo 1 y 2. Sin embargo, pos-ejercicio, el LS incrementó (inicio del estudio 3,8 mmol/L; final del ciclo [1] 5,64 mmol/L; final del ciclo [2] 6,5 mmol/L) (tabla 3). Por su parte, el lactato sanguíneo obtenido inmediatamente al final de las sesiones de natación fue significativamente inferior ($p=0,03$) en el mes 4 que en el mes 1 de entrenamiento (LS mes 1= 2,52 mmol/L y LS mes 4= 0,75 mmol/L).

A pesar de encontrarse diferencias estadísticamente significativas para la concentración sanguínea de sodio, y valor de SID, ATOT y osmolaridad (OSM) después de la natación, no hubo diferencia biológica relevante (tablas 3 y 4), ya que todos los valores se encontraron dentro de los rangos fisiológicos de referencia.

Producción y aclaramiento de lactato durante las pruebas de esfuerzo

La FC2 (frecuencia cardiaca a la cual se alcanza la concentración de lactato sanguíneo de 2 mmol/L o umbral aerobio) y la FC4 (frecuencia cardiaca a la cual se alcanza la concentración de lactato sanguíneo de 4 mmol/L o umbral anaerobio), fueron calculados para cada caballo en cada prueba de esfuerzo. Algunas pruebas no arrojaron valores de LS iguales o superiores a 4 mmol/L de modo que no pudo ser calculado en esos casos. De igual manera, se calculó el porcentaje de aclaramiento del LS durante la etapa de recuperación en todas las pruebas de esfuerzo (tabla 2).

Tabla 2. Umbral anaerobio y aclaramiento de lactato en cada prueba de esfuerzo, expresado individualmente.

	FC2			**FC4**			**Concentración máxima de lactato sanguíneo (concentración 10 min pos-ejercicio)**			**%Aclaramiento**		
	Inicial	Final de ciclo 1	Final de ciclo 2	Inicial	Final de ciclo 1	Final de ciclo 2	Inicial	Final de ciclo 1	Final de ciclo 2	Inicial	Final de ciclo 1	Final de ciclo 2
Caballo 1	77	205	148	150	NC	190	9.8 (3.6)	1.9 (1.0)	5.7 (2.0)	63	47	65
Caballo 2	163	86	155	190	90	190	5.1 (1.9)	13.7 (7.6)	6.4 (2.3)	63	44	64
Caballo 3	75	169	157	170	170	NC	4.3 (3.3)	5.3 (4.9)	2.8 (0.8)	22	8	71
Caballo 4	142	130	136	180	160	170	6.2 (1.7)	5.4 (1.7)	6.2 (1.8)	69	71	71
Caballo 5	129	135	158	NC	NC	NC	1.7 (0.6)	1.1 (0.8)	2.4 (0.7)	65	27	71
Caballo 6	182	196	174	155	180	200	4.2 (1.1)	4.5 (2.0)	4.1 (0.9)	74	56	78

FC2= Frecuencia cardiaca a la cual se alcanzó la concentración sanguínea de lactato de 2 mmol/L; FC4= Frecuencia cardiaca a la cual se alcanzó la concentración sanguínea de lactato de 4 mmol/L; NC= no fue calculado por encontrarse la concentración de lactato máxima <4 mmol/L.

Actividad bioquímica

El análisis de la concentración sanguínea de creatinina, BUN, GGT y FA no arrojó diferencias significativas entre el reposo y el final del ejercicio en todas las pruebas de esfuerzo.

Discusión

El protocolo de entrenamiento en piscina aplicado permitió mejorar la capacidad aerobia en al menos la mitad de los animales utilizados en el estudio, evaluado a partir de la FC4, al observarse una frecuencia cardiaca mayor al alcanzarse el umbral anaerobio. Por otro lado, se evidenció adaptación cardiaca al ejercicio, cuando se analizó la frecuencia cardiaca máxima alcanzada en piscina cada mes (figura 5). Es probable que los animales desarrollaran hipertrofia cardiaca, como ha sido reportado previamente en ratones [8,9].

También fue posible determinar, que este protocolo de entrenamiento en piscina para CPC no afecta los parámetros relacionados con las células sanguíneas de la línea roja y leucocitos; este resultado está relacionado con el descenso programado de la intensidad en cada ciclo de entrenamiento, la intensidad del entrenamiento ha sido reportada antes como factor relacionado con la presentación de anormalidades hemáticas en caballos [10]. El protocolo utilizado en el presente estudio no produjo cambios en el estado ácido-base, tanto en el ejercicio realizado en superficie con jinete como en piscina. Este resultado puede deberse a la adaptación de los mecanismos de termorregulación, que facilitan la conservación de agua y electrolitos corporales, disminuyendo de esa manera la posibilidad de producirse alteración del estado ácido-base. Por otro lado, la mejoría en la capacidad aerobia disminuye la alteración en la presión parcial sanguínea de CO_2, así también la probabilidad de encontrar alteraciones del pH es baja.

Algunas variables medidas mostraron aumento en su concentración debido a la pérdida de agua, vista a través del cálculo del cambio en el volumen plasmático, aunque en ningún caso se registraron valores por fuera del rango fisiológico de referencia.

Uno de los resultados representativos fue la modificación en la producción y aclaramiento del lactato. Durante las pruebas de esfuerzo se encontró que la concentración de LS incrementó, esto inicialmente podría interpretarse como disminución de la capacidad aerobia, sin embargo, para este caso, se pudo establecer que a pesar del aumento en la producción de lactato existió una mejoría en los mecanismos disponibles fisiológicamente para aclararlo después del ejercicio (tabla 2). Es probable que esto se haya logrado por el cambio suscitado en las fibras musculares de contracción lenta.

La concentración de lactato en sangre durante el ejercicio y la recuperación es un fenómeno complejo influenciado por la producción de lactato por parte del músculo en ejercicio, el flujo sanguíneo y la captación y oxidación de lactato por parte del hígado, el corazón y el músculo esquelético [11].

Finalmente, se pudo evidenciar que este tipo de entrenamiento combinado no produce alteraciones a corto o mediano plazo (hasta 4 meses) sobre la actividad enzimática muscular, como se registró en los análisis bioquímicos.

Conclusiones

El entrenamiento de CPC bajo un protocolo de entrenamiento en tierra combinado con natación produce adaptaciones cardiovasculares y metabólicas que aumentan la capacidad aerobia, sin generar alteraciones hematológicas ni bioquímicas asociadas al ejercicio. Por otro lado, la duración del entrenamiento combinado depende del objetivo que haya sido diseñado para cada caballo, cuando se busca adaptación cardiovascular o conversión de masa es necesario mantener el programa durante al menos 4 meses, si por otro lado se busca desplazar el umbral aerobio y anaerobio, se prefiere mantener el entrenamiento durante dos meses y ajustar la intensidad para el entrenamiento para los meses posteriores.

Agradecimientos

Al centro equino Normandía por facilitar sus instalaciones, animales y personal calificado.

Tabla 3. Parámetros hematológicos de caballos de paso colombiano (CPC) al inicio, 2 meses y 4 meses de un protocolo de entrenamiento en piscina.

		inicial			final del ciclo 1			fin de ciclo 2		
		Me	DS	RIC	Me	DS	RIC	Me	DS	RIC
Hematocrito (%)	Reposo	39,6	5,1	36,5-43,8	42,2	14,1	32,9-45,6	42	40,8	36,5-42,6
	Pos-ejercicio	45,9	7	42,9-50,6	50,4	5,5	49-54,8	51,9	40,1	50,4-52,9
Hemoglobina (g/dL)	Reposo	13,8	2	11,9-14,2	13,9	1,7	12,3-14,7	14,2	1,4	12,4-14,7
	Pos-ejercicio	15,3	1,7	14,8-15,8	16	2,3	14,8-16,6	17,2	1,3	17-17,9
CMHC (g/dL)	Reposo	33,9	1,2	32,5-34,8	31,8	1,9	30,3-33,5	34,1	0,5	33,9-34,5
	Pos-ejercicio	33,2	1,9	31,7-35,1	31,1	2,1	29,4-33,2	33,8	0,4	33,5-33,8
proteínas totales (g/dL)	Reposo	6,6	0,5	6,08-6,64	6,3	0,3	6,29-6,42	6,7	0,2	6,6-6,8
	Pos-ejercicio	6,7	0,7	6,1-7,13	6,8	0,2	6,74-6,88	6,9	0,3	6,8-7,1
fibrinógeno (mg/dL)	Reposo	450	225,8	200-700	300	109,5	200-400	300	297,4	200-800
	Pos-ejercicio	450	234,5	200-600	300	109,5	200-400	200	242,2	200-400
albúmina (g/dL)	Reposo	3,5	0,3	3,31-3,87	3,4	0,2	3,3-3,56	3,8	0,6	3,7-4,8
	Pos-ejercicio	3,7	0,3	3,62-4,01	3,6	0,3	3,41-3,72	4	0,5	3,7-4,7
leucocitos (x 103/mm3)	Reposo	7,6	1,9	7,1-8,2	7,4	2,2	6,9-9,9	7,1	1,9	6-sep
	Pos-ejercicio	8,9	2,2	7,8-9,5	8,2	1,9	8,1-10,9	9,5	16,9	7,8-11,4
neutrófilos (x 103/mm3)	Reposo	3,7	1	3,11-4,02	3,6	2,3	3,17-6,33	3,4	0,9	3,21-4,86
	Pos-ejercicio	3,4	1,1	2,68-4,83	3,7	1,6	3,15-4,79	3,8	0,9	3,36-4,95
linfocitos (x 103/mm3)	Reposo	4,5	1,6	3,2-5,18	3,1	0,8	2,59-3,85	3,4	1,7	2,76-3,87
	Pos-ejercicio	4,9	2,2	4,02-5,58	4,4	1,5	3,81-5,38	4	1	3,6-5,47
eosinófilos (x 103/mm3)	Reposo	0,1	0,1	0,07-0,16	0,1	0,2	0,06-0,34	0,2	0,1	0,07-0,18
	Pos-ejercicio	0,3	0,3	0,09-0,39	0,2	0,3	0,08-0,32	0,1	0,2	0-0,24
monocitos (x 103/mm3)	Reposo	0	0,1	0-0,11	0	0,1	0-0,09	0,1	0,1	0,09-0,18

	Pos-ejercicio	0	0	0-0,07	0	0,1	0-0,1	0,3	0,1	0,22-0,34
basófilos (x 103/mm3)	Reposo	0	0	0	0	0	0-0	0	0	0
	Pos-ejercicio	0	0	0	0	0	0	0	0,1	0-0,11
bandas (x 103/mm3)	Reposo	0	0,1	0-0,14	0	0	0	0	0	0
	Pos-ejercicio	0	0,1	0-0,07	0	0,1	0	0	0	0
plaquetas (x 103/mm3)	Reposo	265	70,4	221-341	300,5	39,7	295-312	205,5	54,8	167-267
	Pos-ejercicio	274,5	55,4	202-317	309,5	27,7	282-334	216	63	154-278

Me= mediana; DS= desviación estándar; RIC= rango intercuartil.

Tabla 4. Parámetros ácido-base y electrolíticos medidos en caballos de paso colombiano (CPC) al inicio, 2 meses y 4 meses de un protocolo de entrenamiento en piscina.

		Inicial			Fin del ciclo 1			Fin del ciclo 2		
		Me	DS	RIC	Me	DS	RIC	Me	DS	RIC
Sodio (mmol/L)	Reposo	137	2,6	135-139	137	1,63	136-138	136,5	1,94	136-139
	Pos-ejercicio	137	1,064	136-139	137	1,5	136-138	134,2	2,07	134-136
Cloro (mmol/L)	Reposo	103	1,37	103-103	104	1,37	103-106	105	2,43	104-106
	Pos-ejercicio	102	1,6	101-104	103	1,97	102-105	105,5	0,98	105-107
Potasio (mmol/L)	Reposo	4,2	0,43	4,2-4,7	4,5	0,4	4,4-4,8	4,5	0,71	4-4,5
	Pos-ejercicio	3,85	3,88	3,7-4,1	4,05	0,46	3,9-4,3	4,15	0,57	3,8-4,8
Lactato (mmol/L)	Reposo	1,09	0,185	0,73-2,79	0,66	0,3	0,36-0,98	0,895	0,41	0,64-1,04
	Pos-ejercicio	3,8*	2,01	1,85-4,52	5,64*	2,6	2,08-7,33	6,5*	1,96	4,43-7,36
Bicarbonato (mmol/L)	Reposo	24,9	0,63	24,6-25,3	25,8	1,39	25,5-26,8	23,45	0,76	23,2-24
	Pos-ejercicio	24,6	3,6	22,8-26,1	21,75	1,99	20,7-23	18,25	1,9	18,1-20,9
Glucosa (mg/dL)	Reposo	90,5	12,94	85-99	104,5	90,5	96-112	100,5	9,35	98-106
	Pos-ejercicio	114	22,39	101-124	120	13,67	111-133	116	7,83	112-127
Calcio (mmol/L)	Reposo	1,475	0,13	1,47-1,6	1,47	0,175	1,32-1,61	1,46	0,13	1,24-1,47

	Pos-ejercicio	1,39	0,075	1,37-1,47	1,38	0,134	1,3-1,51	1,14	0,23	0,97-1,37
pH	Reposo	7,41	0,048	7,4-7,46	7,44	0,026	7,43-7,44	7,44	0,042	7,4-7,45
	Pos-ejercicio	7,41	2,43	7,41-7,44	7,48	0,037	7,44-7,48	7,46	0,028	7,44-7,48
BE	Reposo	0,55	0,97	0,4-1,3	1,75	1,73	1,3-2,4	-0,7	0,87	-1,3-0,1
	Pos-ejercicio	0,75	3,39	-1,1-1,3	-2,25	1,86	-2,7-(-1,74)	-5,6	1,84	-5,7-(-2,6)
SID	Reposo	36,37	1,64	34,97-37,71	36,89	2,62	34,5-39,44	35,14	1,29	34,86-35,81
	Pos-ejercicio	36,51	3,07	33,85-37,86	32,5	2,9	30,78-35,02	26,8	3,96	23,22-29,56
ATOT	Reposo	14,62	1,08	13,61-14,94	14,49	0,42	14,17-14,87	14,5	0,75	14,18-15,28
	Pos-ejercicio	14,62*	0,93	13,66-15,41	15,57*	0,69	15,09-15,97	15,41*	0,81	15,25-15,95
VOL		6,98	9,16	-1,49-9,23	4,67	8,07	-6,32-7,23	-1,19	6,26	-2,49-1,94

Me= mediana; DS= desviación estándar; RIC= rango intercuartil;*= diferencia estadísticamente significativa entre ciclos. valor p≤0.1

Tabla 5. Parámetros ácido-base y electrolíticos en caballos de paso colombiano (CPC) durante la natación, en reposo y posterior a una sesión de entrenamiento en el mes 1 y mes 5.

		Mes 1			Mes 5		
		Me	DS	RIC	Me	DS	RIC
Sodio							
(mmol/L)	Reposo	136*	0,84	135-136	137,5*	1,52	137-139
	Pos-ejercicio	136*	2,17	134-137	138*	1,72	137-139
Cloro							
(mmol/L)	Reposo	106,5	4,07	103-109	106,5	3,31	103-108
	Pos-ejercicio	107	3,97	101-109	105,5	3,54	104-106
Potasio							
(mmol/L)	Reposo	4,15	0,39	3,9-4,4	4,5	0,46	4,3-5
	Pos-ejercicio	4,25	0,18	4,2-4,4	4,4	0,4	4-4,8
Lactato							
(mmol/L)	Reposo	0,53[a]	0,14	0,43-0,62	0,56	0,35	0,43-0,71
	Pos-ejercicio	2,52[a]*	4,2	0,55-7,33	0,75*	3,03	0,48-6,13
Bicarbonato							
(mmol/L)	Reposo	25,75	1,14	25,3-26,9	25,25	1,24	23,9-26,2
	Pos-ejercicio	24,3	4,08	20,5-26,3	24,6	3,18	22,8-27,2
Glucosa	Reposo	103,5	14,8	100-110	101	7,5	95-110

(mg/dL)							
	Pos-ejercicio	97	12,9	80-100	88,5	16,3	84-95
Calcio							
(mmol/L)	Reposo	1,32	0,17	1,3-1,62	1,5	0,077	1,45-1,51
	Pos-ejercicio	1,4	0,17	1,23-1,52	1,46	0,044	1,45-1,53
pH	Reposo	7,43	0,021	7,41-7,44	7,4	0,031	7,4-7,44
	Pos-ejercicio	7,47	0,054	7,39-7,49	7,43	0,036	7,4-7,46
BE	Reposo	1,5	1,06	0,7-2	0,65	1,39	-0,3-2
	Pos-ejercicio	-0,15	4,7	-2,8-3,0	0,3	3,65	-2,4-3,4
SID	Reposo	32,86[a]	4,36	29,94-36,78	35,24[b]	3,41	32,69-38,77
	Pos-ejercicio	30,64[a]	7,19	24,64-35,55	35,85[b]	5,88	30,77-36,53
OSM	Reposo	272*	1,7	270-272	275*	3,03	274-278
	Pos-ejercicio	272	4,33	268-274	276	3,44	274-278

Me= mediana; DS= desviación estándar; RIC= rango intercuartil; *= diferencia estadísticamente significativa entre el mes 1 y mes 5. valor p ≤0.1; [a]= diferencia estadísticamente significativa entre el reposo y pos-ejercicio. valor p ≤0.1

Referencias bibliográficas

1. Arias, M.P.; Maya, J.S.; Arango, L. (2019) Efectos de dos protocolos de entrenamiento sobre el lactato sanguíneo en caballos de paso fino. Rev. la Fac. Med. Vet. y Zootec. 66, 219–230. https://doi.org/10.15446/rfmvz.v66n3.84259
2. Boffi, F. (2007) Fisiología del ejercicio en equinos, 1st ed., Intermédica.
3. Bonelli, F.; Sgorbini, M.; Meucci, V.; Sighieri, C.; Baragli, P. (2017) How swimming affects plasma insulin and glucose concentration in Thoroughbreds: A pilot study. Vet. J. 226, 1–3. https://doi.org/10.1016/j.tvjl.2017.06.006
4. Evangelista, F.S.; Brum, P.C.; Krieger, J.E. (2003) Duration-controlled swimming exercise training induces cardiac hypertrophy in mice. Brazilian J. Med. Biol. Res. 36, 1751–1759. https://doi.org/10.1590/S0100-879X2003001200018
5. Garcia, M.C.; Beech, J. (1986) Endocrinologic, hematologic, and heart rate changes in swimming horses. Am. J. Vet. Res. 47, 2004–2006. https://pubmed.ncbi.nlm.nih.gov/3532876/
6. Greenwood, J.D.; Moses, G.E.; Bernardino, F.M.; Gaesser, G.A.; Weltman, A. (2008) Intensity of exercise recovery, blood lactate disappearance, and subsequent swimming performance. J. Sports Sci. 26, 29–34. https://doi.org/10.1080/02640410701287263
7. Henneke, D.R.; Potter, G.D.; Kreider, J.L.; Yeates, B.F. (1983) Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15, 371–372. https://doi.org/10.1111/j.2042-3306.1983.tb01826.x

8. Jones, S.; Franklin, S.; Martin, C.; Steel, C. (2020) Complete upper airway collapse and apnoea during tethered swimming in horses. Equine Vet. J. 52, 352–358. https://doi.org/10.1111/evj.13177

9. Kupczyński, R.; Śpitalniak, K.; Zwyrzykowska-Wodzińska, A.; Soroko, M. (2018) The influence of different workload trainings on some blood parameters in show jumping horses. Vet. Arh. 88, 279–293. https://doi.org/10.24099/vet.arhiv.170513

10. Medeiros, A.; Oliveira, E.M.; Gianolla, R.; Casarini, D.E.; Negrão, C.E.; Brum, P.C. (2004) Swimming training increases cardiac vagal activity and induces cardiac hypertrophy in rats. Brazilian J. Med. Biol. Res. 37, 1909–1917. https://doi.org/10.1590/S0100-879X2004001200018

11. Zamparo, P., Cortesi, M. and Gatta, G. (2020) The energy cost of swimming and its determinants. Eur. J. Appl. Physiol. 120, 41–66. https://doi.org/10.1007/s00421-019-04270-y

Conclusiones generales

1. Las respuestas fisiológicas al ejercicio en caballos de paso colombianos (CPC o CCC) con sobrepeso y obesos son similares a las que han sido reportadas para otras razas de caballos. Tales respuestas son hemoconcentración y cambio negativo del volumen plasmático, aumento de la frecuencia cardiaca y aumento en la producción de lactato sanguíneo. Además, se encuentra respuesta de estrés anticipada al ejercicio.
2. La natación para caballos es un ejercicio de alta y máxima intensidad. Los CPC experimentan estrés durante las primeras sesiones de entrenamiento y requieren de un proceso de adaptación mínimo de una semana para conseguir entrenar sin riesgos de fatiga aguda.
3. Los caballos desentrenados evidencian la pérdida de la condición atlética a través del cálculo del umbral anaerobio. Al no encontrarse una tendencia en la producción de lactato sanguíneo, se pudo concluir que el umbral anaerobio es individual para cada animal y no para la raza.
4. El protocolo de entrenamiento en piscina para CPC en sobrepeso y obesos, produce adaptaciones cardiovasculares y metabólicas y ninguna alteración hematológica o bioquímica. Las adaptaciones logradas fueron disminución de la frecuencia cardiaca tanto en reposo como máxima, además, mejoró el proceso de aclaramiento del lactato sanguíneo.
5. El protocolo de entrenamiento en piscina para CPC en sobrepeso u obesos facilita la movilización de tejido adiposo, la conversión de masa y además aumenta la sensibilidad a la insulina.
6. La prueba de esfuerzo en campo para CPC es útil en la verificación de los avances en el entrenamiento.
7. El consumo de oxígeno no debe ser calculado en función de la frecuencia cardiaca, a menos que el caballo se ejercite a intensidad submáxima. De lo contrario, muchos

factores intervienen en el proceso de intercambio gaseoso. Por ejemplo: alteración de la afinidad de la hemoglobina por el oxígeno o hiperviscosidad sanguínea.

Recomendaciones

En el desarrollo de la investigación en tópicos relacionados con acondicionamiento y entrenamiento para caballos se debe utilizar herramientas de medición que sean precisas y confiables, especialmente cuando se utiliza la natación, puesto que los animales alcanzan con facilidad zonas de intensidad que mantenidas por largos periodos de tiempo pueden producir fatiga, daño muscular, fibrilación atrial e incluso colapso.

En ningún caso debe aplicarse el entrenamiento en piscina a los caballos en ausencia de herramientas de monitoreo de la frecuencia cardiaca y el lactato sanguíneo.

Cada caballo en entrenamiento en piscina debe ser valorado también durante una sesión de entrenamiento o prueba de esfuerzo de campo con el fin de encontrar efectos del programa sobre la actividad competitiva que desempeña y de esa forma hacer los ajustes que mejor funcionan para cada animal en particular.

Debe considerarse aumentar el número de caballos que sean sometidos a protocolos de natación para lograr extrapolar los resultados a la raza, ya que el grupo de caballos que fue evaluado en este estudio fue reducido.

Por otro lado, es de interés para el país continuar con el estudio de la fisiología del ejercicio y los efectos de diversos tipos de entrenamientos en CPC, puesto que la información continúa siendo escasa.

Anexos

UNIVERSIDAD DE ANTIOQUIA

Vicerrectoría de Investigación

Comité de Ética para la Experimentación con Animales

Medellín, 07 de febrero de 2019

Investigadora
Angélica María Zuluaga Cabrera
Grupo de investigación "CENTAURO"
Universidad de Antioquia

Proyecto*: "Caracterización clínica, endocrina y metabólica de caballos criollos colombianos obesos sometidos a protocolos de natación"*

Resultado de la revisión: Otorgar aval

Cordial saludo.

Luego de estudiada su solicitud para el protocolo de la referencia, el **Comité de Ética para la Experimentación con Animales** le expresa que, se otorgar el aval ético solicitado, tal y como constará en el acta de sesión N° 122 del 05 de febrero.

Con toda atención.

JOSÉ IGNACIO CALLE POSADA
Coordinador
Comité de Ética para la Experimentación con Animales
Universidad de Antioquia

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REVISTA MEXICANA DE CIENCIAS PECUARIAS

Ciudad de México a 15 de junio de 2021.

Angélica María Zuluaga Cabrera, María José Casas Soto, José Ramón Martínez Aranzales, Viviana Elena Castillo Vanegas, Nathalia María del Pilar Correa Valencia, María Patricia Arias Gutiérrez

ID: 5882

Estimados autores:

Tengo el agrado de comunicar a ustedes que su trabajo titulado: **"Hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian paso horses"** con ID: 5882 ha sido aceptado por nuestro cuerpo arbitral como artículo científico.

Le sugerimos que estén atentos a nuestros futuros comunicados para informarles sobre los avances del proceso editorial de su trabajo.

A T E N T A M E N T E

MVZ. Arturo García Fraustro
Editor en Jefe.

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RESULTADO DA AVALIAÇÃO

O trabalho intitulado "LIMIAR ANAERÓBIO EM CAVALOS DA RAÇA PASSO COLOMBIANO SUBMETIDOS A PROVA DE ESFORÇO A CAMPO" foi **APROVADO** no evento **X Simpósio Internacional do Cavalo Atleta - SIMCAV 2021**

- **Título:** LIMIAR ANAERÓBIO EM CAVALOS DA RAÇA PASSO COLOMBIANO SUBMETIDOS A PROVA DE ESFORÇO A CAMPO
- **Número:** 332209
- **Data de Submissão:** 11/03/2021
- **Modalidade:** Resumo simples
- **Área Temática:** Estudo Científico - Fisiologia do exercício, Treinamento, Reabilitação e Fisioterapia
- **Autores:** Angélica María Zuluaga Cabrera,María J. Casas-Soto,JOSE RAMON MARTINEZ ARANZALES,Nathalia M. del P. Correa-Valencia,María P. Arias-Gutiérrez

IX SIMPÓSIO INTERNACIONAL DO CAVALO ATLETA
XIV FÓRUM DE GASTROENTEROLOGIA EQUINA

25, 26 e 27 de Abril de 2019- Belo Horizonte/MG

Escola de Veterinária UFMG

EQUINOVA Ciência a serviço do cavalo

CERTIFICADO

Certificamos que **Angélica M. Z. Cabrera, Maria P.A. Gutierrez, José R. Martínez A.** apresentaram o trabalho em pôster intitulado *"RESPOSTA CARDÍACA DE UM CAVALO CRIOULO COLOMBIANO AO EXERCÍCIO EM PISCINA: RELATO DE CASO"*, durante o **IX Simpósio Internacional do Cavalo Atleta** e **XIV Fórum de Gastroenterologia Equina**, na Universidade Federal de Minas Gerais de 25 a 27 de abril de 2019.

Belo Horizonte/MG, 27 de Abril de 2019.

PROF. ARMANDO DE MATTOS CARVALHO COORDENADOR	PROFA. ANDRESSA SILVEIRA XAVIER COORDENADOR	PROF. GERALDO ELENO SILVEIRA ALVES COORDENADOR	PROF. RAFAEL RESENDE FALEIROS COORDENADOR

Aceptación de resumen

Encuentro de Investigadores de las Ciencias Precuarias Universidad de Antioquia <enicip@udea.edu.co>
para mí

Buenas tardes,

El comité evaluador informa que el resumen titulado " Efecto de un protocolo de natación sobre la grasa corporal, hormonas y actividad muscular en caballos de paso colombianos* " fue aceptado para presentar en el ENICIP 2021.

Cordialmente,
Luz Victoria Orozco

Aceptación de resumen

Recibidos

Encuentro de Investigadores de las Ciencias Precuarias Universidad de Antioquia <enicip@udea.edu.co>
para mí

lun, 10 may 12:56 (hace 9 días)

Buenas tardes,

El comité evaluador informa que el resumen titulado " Hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian paso horses " fue aceptado para presentar en el ENICIP 2021.

Cordialmente,
Luz Victoria Orozco

UNIVERSIDAD DE ANTIOQUIA
Facultad de Ciencias Agrarias

Date:	14 Oct 2021
To:	"Angélica María Zuluaga Cabrera" angelica.zuluaga@udea.edu.co
From:	"CEP Journal Office" cepadmin@wageningenacademic.com
Subject:	Decision for CEP-210024R1

Dear Dr. Zuluaga Cabrera,

I am pleased to inform you that your manuscript CEP-210024R1 entitled "Body mass conversion and improved insulin response in Colombian Paso Horses (CPHs) subjected to a swimming training program." is accepted for publication in Comparative Exercise Physiology, under condition that no problems arise during the editing stage at the publisher. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter. The manuscript was accepted on 14 Oct 2021.

Note that that the publisher needs a signed Copyright Transfer Agreement (CTA) from the first or the corresponding author before the manuscript can be published. Please fax (+31 317 453417) or send the CTA by email to the publisher (cep@wageningenacademic.com) as soon as possible if you have not done already. A blank CTA can be found at: www.WageningenAcademic.com/pb-assets/wagen/files/cep/CTA%20Comparative%20Exercise%20Physiology.doc

I also would like to draw your attention to the possibility of publishing your manuscript as 'open access' for 1800 Euro in Comparative Exercise Physiology. With open access, your article will be free available online for everyone. Open access publishing significantly increases the exposure and citation of your work. Often research grants have funding available for dissemination of the results of the research.

Thank you for your fine contribution. On behalf of the Editors of the Comparative Exercise Physiology, we look forward to your continued contributions to the Journal.

With kind regards,

Kenneth Harrington McKeever, PhD, FACSM, FAPS
Editor in Chief
Comparative Exercise Physiology

Comments from the Editors and Reviewers:

Reviewer #1: The work was reviewed and the requests recommended by the reviewer were included. Improved tables like this with extra requested information included and appropriate to the text. The article can be accepted for publication.

Reviewer #2: Thank you for your answers and corrections made to the manuscript.
