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ORIGINAL ARTICLE

Influence of selenium and vitamin E supplementation on energy metabolism in horses used in policing activity

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Abstract This study evaluated the effect of supplementation with selenium and vitamin E on some blood variables in 11 adult male horses used in policing activity. Each animal was treated with 2.8 mg/400 kg of selenium and 2.000 UI/400 kg of vitamin E of body weight, orally, for a period of 30 days. On days 0 (before) and 30 (after), the animals were assessed, and blood samples were obtained before and after exercise. Glucose and lactate and plasma cortisol and serum insulin were determined. On day 0, serum cortisol concentrations were 56.8±12.1 ng/ml and 43.4±19.2 ng/ml, plasma lactate were 6.2±0.7 mg/dl and 12.3±9.2 mg/dl, serum glucose levels were 69.2±5.2 mg/dl and 77.5±6.6 mg/dl, and serum insulin were 3.1±5.1 μUI/ml and 1.5±1.6 μUI/ml, respectively, before and after exercise (M1 and M2). On day 30, serum cortisol concentrations were 55.6±15.6 ng/ml and 27.6±12.2 ng/mL, plasma lactate were 7.0±0.9 mg/dl and 8.4±1.7 mg/dl, plasma glucose were 76.0±4.1 mg/dl and 77.1±8.9 mg/dl, and serum insulin were 3.3±6.9 μUI/ml and 2.1±3.7 μUI/ml, respectively, before and after exercise (M1S and M2S). As a result of the exercise, it was shown a reduction in serum insulin and an increase in plasma lactate and glucose. Supplementation with selenium and vitamin E resulted in decreased levels of cortisol and lactate after physical activity, possibly indicating that supplementation contributed to better utilization of plasma glucose and improved adaptation to physical exercise.

Keywords Equine · Biochemistry · Selenium · Vitamin E · Exercise

Introduction

Facing a large variety of function, the horses are fed and exercised in order to achieve the best results. The athletic horse has great ability to produce work reflecting in a body capable of storing and producing high amount of energy (Etchichury 2004; Casella et al. 2012). However, Powers et al. (1999) demonstrated that increasing the metabolic rate of the muscles during exercise results in increased production of free radicals.

According to Ji (1999) and Etchichury (2004), supplementation of enzymatic and non-enzymatic antioxidants has shown great efficacy in individuals in adapting to acute and chronic exercise. The delicate balance between pro-oxidants and antioxidants suggests that supplementation may be desirable for physically active individuals and adapted to exercise, thus generating a higher margin of protection to oxidative stress (Etchichury 2004).

Vitamin E is the main antioxidant biologically associated with oxidative stress caused by exercise. Besides, selenium has also been used as a supplement for making part of the glutathione's system, a key antioxidant enzyme mechanism (Williams and Carlucci 2006; Dias et al. 2009).

Many beneficial effects are attributed to selenium and vitamin E, but in fact, there are few studies that confirm that such supplementation is beneficial for sport horses; also, the relationship between nutrition and myopathies is not well understood (Lewis 2000).

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Dias et al. (2009) states that selenium present in glutathione peroxidase and vitamin E have a complementary action in protecting cell membranes, preventing peroxidation injuries in muscle. Further asserts that both act to protect membrane's lipids but in different ways: vitamin E preventing unsaturated lipid oxidation and interrupting the effect of free radicals, and selenium destroying peroxides derived from cellular metabolism.

Takanami et al. (2000) recommends vitamin E supplementation as a way of preventing oxidative damage resulting from the exercise. According to Pagan et al. (1999), it is important to consider that prolonged exercise increases the glomerular renal filtrate thus increasing selenium excretion via urine, less intense effect that in case of selenium supplementation. Dias et al. (2009) argues that the administration of selenium can increase the horse exercise performance.

One of the models used to study the effect of antioxidant supplementation in the diet of human athletes is by evaluating its effect on the blood constituents and markers of muscle activity and energy (Vazquez et al. 2006) also in sport horses in field tests (Williams and Carlucci 2006) and experimental conditions (Silveira 2005).

Exercise can be defined as "normal stress" stimulating body functions (Lessa 2003). According to Leal et al. (2007) and Gontijo et al. (2014), policing animals are stabled, and it provokes changes in cortisol levels consistent with a situation of chronic stress. The same author states that horses subjected to urban patrol activities have their welfare compromised in relation to animals kept in paddocks without that activity. These animals have a greater chance of developing abnormal behavior and colic. Cortisol is a major glucocorticoid secreted by the adrenal cortex of the horses, which is involved in the regulation of plasma glucose concentration, increasing its concentration when it is released. The secretion of cortisol is related to anaerobic activity; it is therefore suggested that plasma lactate concentration is a stimulator of cortisol (Leal et al. 2007).

The concentration of blood or serum lactate has been used as frequently as the clinical parameters and provides additional information about the current fitness of the athlete (Lindner 2000). According to Lessa (2003), reports suggest lactate as a stimulator of cortisol secretion. The investigation of the relationship between lactate and cortisol in horses training is important to assess the degree of exercise and stress of animals subjected to policing activity.

The increase in plasma glucose is a common response to stress (Mckeever 2002; Mcgowan 2008) and is an extra source of energy that allows the animal (Moreira et al. 2015) to overcome the disturbances caused by the stressor (Matteri et al. 2000). Fernandes and Larsson (2000) reported that horses submitted to exercise, more specifically an endurance race of 30 km, may have significant increases in serum glucose, urea, and sodium. Ribeiro et al. (2004) observed that horses subjected to endurance tests also showed elevations in serum glucose.

Insulin is a hormone secreted when there is an increase in plasma concentrations of glucose and serves to enhance the cellular uptake and lipogenesis. Horses also respond to agents that stimulate insulin secretion such as arginine and lysine, similar to that observed in humans (Ralston 2002). According to McKeever (2002), insulin response to intense exercise has been well documented in the horse. The horse, like humans and other species, suppresses insulin during exercise. Functionally, this allows the animal to increase gluconeogenesis to maintain blood glucose in appropriate concentrations during exercise. Glucose mobilized during exercise can be used by muscles once connected to insulin; however, performance in endurance tests, for example, seems to be limited by fatigue central mechanisms than the periphery.

The aim of this study was to evaluate the influence of selenium and vitamin E supplementation on energy metabolism in horses submitted to the urban policing activity. The hypothesis tested was that the supplementation of selenium and vitamin E improve the energetic metabolism of horses submitted to exercise.

Material and methods

Eleven male horses of Brasileiro de Hipismo breed, aged between 10 and 20 years, were used in the experiment. In terms of housing, feeding, and hygienic management, all animals were kept under similar conditions. The animals received daily Tifton sp. hay ad libitum followed by a commercial diet (1.2 kg/100 kg of BW—Royal Horse SPORT—Socil Evialis Nutrition Animal and supplemented with 100 g of inorganic mineral (Tortuga Zootécnica Land Company Ltda.)).

Age, weight, general health, attitude, appearance of mucous membranes, and physiological parameters of each animal were recorded. The animals were considered healthy on physical examination, and according to the parameters suggested by Radostits et al. (2007).

All horses selected for the experiment were used to the policing activity imposed, and they have been practicing it for at least 6 months prior the experiment. Animals left the stables for work either in the morning and had a working shift of 8 h per day. The type of exercise was classified as moderate—low intensity and long duration—in which the horses switched periods of trot and walk. All horses were submitted to the same type of exercises and went for the work in the same group, before and after supplementation.

Four blood samples were taken from each animal. Two were taken before the supplementation period, and this was considered the control values. M1 was obtained at rest in individual stalls, with the animals fastingfor 8 h, and M2 was obtained immediately after 8 h of exercise. Both moments were without supplementation. A dosage of 2.8 mg/400 kg of selenium and 2.000 UI/400 kg of vitamin E of body weight orally were added for 30 days. These dosages were higher than the guidelines of the National Research Council (2007). M1S was obtained at rest in individual stalls, with the animals fasting for 8 h, and M2S was obtained immediately after 8 h of exercise, both data after 30 days of supplementation. During the time of supplementation, no horses had health problems.

Blood samples were obtained after local antisepsis from the *vena jugularis externa* with disposable needles for multiple samples (25 mm × 0.8 mm), using a vacuum system (Vacutainer®). For the determination of plasma lactate and glucose, glass tubes containing fluoride sodium were used. For the determination of serum cortisol and insulin, siliconized glass tubes without anticoagulants were used. Analyses were performed in a semi-automatic biochemical analyzer (BioPlus 2000, Quimis Scientific Instruments Ltd, Diadema, SP, Brazil) according to the manual of the commercially available kit (Bioclin—Quibasa, Basic Chemistry, Belo Horizonte, MG, Brazil) in the Laboratório Clínico Veterinário do Centro Universitário Vila Velha. The determination of serum cortisol and insulin was performed in the BET Laboratories, Rio de Janeiro, RJ, using the technique of solid phase radioimmunoassay (Gamma Count Cobra 2—DPC MedLab—Sao Paulo, SP, Brazil) and commercially available kits (DPC MedLab—Sao Paulo, SP, Brazil) according to the manual. All tests were performed twice.

The gathered data was analyzed using the program SAEG (SAEG/UFV, 2007). The analysis of variance at 5 % probability was used to identify possible differences between the variances of the treatments, and the Student *t* test, also at 5 % probability, was used to detect differences between the treatments.

Results and discussion

The results of biochemical and hormonal analysis are shown as mean±standard deviation in Table 1. Biochemical analysis

Table 1 Results of biochemical and hormonal parameters before and after supplementation with selenium and vitamin E in horses submitted to physical exercise

Parameter	Before supplementation		After supplementation	
	M1	M2	M1S	M2S
Cortisol (ng/ml)	56.8±12.1^{a}*	43.4±19.2^{a}	55.6±15.6^{a}	27.6±12.2^{b}
Lactate (mg/dl)	6.2±0.7^{b}	12.3±9.2^{a}	7.0±0.9^{b}	8.4±1.7^{b}
Glucose (mg/dl)	69.2±5.2^{b}	77.5±6.6^{a}	76.0±4.1^{a}	77.1±8.9^{a}
Insulin (μUI/ml)	3.1±5.1^{a}	1.5±1.6^{b}	3.3±6.9^{a}	2.1±3.7^{a}

Source: Elaboration of the authors

*Within a row, means without a common letter differ ($P<0.05$)

before supplementation with selenium and vitamin E showed significant difference between M1 and M2 for all variables evaluated, except for cortisol.

According to Teixeira-Neto et al. (2007) and Fonseca et al. (2011), the interpretation of cortisol measurements should be done with caution as results may be influenced by other facts despite physical exercise. According to reports of Irvine e Alexander (1994) and Teixeira et al. (2008), the circadian rhythm of cortisol in horses has a peak in the morning and then constantly decreases until evening. Therefore, Linden et al. (1990) and Marc et al. (2000) stated that the determination of plasma cortisol in horses is important to evaluate the stress induced by exercise.

A slight variation in cortisol may be resulting from an adaptation of animals to physical exercise. This was described by Nogueira et al. (2002), who reported that cortisol, lactate, and creatinine vary according to age and physical fitness. Nogueira's study (2002) also mentioned that a reduction in cortisol observed in Thoroughbred 2–3-year-old horses occurred due to better physical conditioning gained after a long period of training, when compared with 1–2-year-old horses that were not exercised and were kept on pasture. The same results were observed in the present experiment, suggesting adaptation to the imposed activity.

There was a rise in plasma lactate when comparing M1 and M2. Gomide et al. (2006) argued that a significant increase in blood lactate occurs only after the end of a demanding physical activity with the main energy being produced anaerobically. The mean values of lactate found in M1 and M2 are similar to those of Fonseca et al. (2011) who stated that the accumulation of lactate is related to the workload and the speed with which animals move during the exercise. This information is also confirmed by Delesalle et al. (2007) who stated that under normal conditions, most lactate is produced by the erythrocytes, but during exercise or strenuous physical activity, the muscle produces large amounts of lactate due to the condition of insufficient oxygenation.

Linden et al. (1990) described a linear relationship between plasma lactate, glucose, and speed of the exercise indicating that aerobic training can minimize hyperlactacidemia. According to Ferraz et al. (2008), the lactate threshold negatively correlates with the plasma glucose. The authors suggested these parameters as indicators of aerobic capacity in horses. Linden et al. (1990) reported that lactate production is much more related to the intensity of exercise than cortisol.

The variation of plasma glucose found in this study is similar to the findings of McKeever (2002), McGowan (2008), Fonseca et al. (2011), and Moreira et al. (2015) who reported that increased plasma glucose is a common response to stress and is an extra source of energy that allows the animal to overcome the disturbances caused by the stressor, regardless of aerobic or anaerobic production of energy.

The results of serum insulin found in this study at the time before supplementation are consistent with those obtained by

McKeever (2002) and Fonseca et al. (2011) who stated that the horse suppresses insulin during exercise. As mentioned above, it allows the animal to increase gluconeogenesis to maintain blood glucose in appropriate concentrations during exercise, and this happens due to the influence of cortisol and cathecolamines. According to Frank (2005), an animal that has high concentrations of plasma cortisol can have a metabolic process known as insulin resistance, during which there is a malfunction of insulin receptors in tissues and higher values of it will be seen. This was not observed in this study, where the suppression of insulin along with a lower level of cortisol after exercise was possible to observe.

Biochemical analysis after supplementation with selenium and vitamin E showed no significant difference between M1S and M2S in the plasma lactate, glucose, and serum insulin. These results agree with those of Sen and Packer (2000) who suggested that supplementation with antioxidants provides a beneficial effect against tissue damage induced by exercise, decreasing plasma lactate. However, significant difference between M1S when compared to M2S was observed for the determination of serum cortisol. Although there is no significant difference for determination of plasma glucose between M2 and M2S, it was possible to observe a significant decrease in plasma lactate and serum cortisol and insulin levels between the same moments. This result is according to Silveira (2005) who suggested that supplementation with vitamin E are effective in protecting cell membranes preventing the spread of lipid peroxidation, thereby helping in the reduction of serum cortisol. Although El-Deeb and El-Bahr (2010) claimed that supplementation of selenium and vitamin E alters the rate of glucose and lactate metabolism in horses, this was not observed in the present research once that plasma glucose and lactate levels were similar between the two moments of analysis. Better energy utilization was probably due to mobilization of other resources like fat and protein mobilization, but they were not evaluated.

Conclusions

Selenium and vitamin E supplementation contributed to the decrease of the average values of cortisol and lactate after the exercise, suggesting a better utilization of energy resources.

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Compliance with ethical standards This study was approved by the Committee on Bioethics of FMVZ-UNESP, Botucatu/SP and was conducted in accordance with the technical standards of biosafety and ethics.

References

Casella S, Alberghina D, Giannetto C, Piccione G (2012) Responses to training and standardised exercise test in the athlete horse: changes in blood gas profile. Comp Clin Pathol 21(2):611–614

Delesalle C, Dewulf J, Lefebvre RA, Schuurkes JAJ, Proot J, Lefere L, Deprez P (2007) Determination of lactate concentrations in blood plasma and peritoneal fluid in horses with colic by an accusport analyzer. J Vet Intern Med 21:293–301

Dias DCR, Rocha JS, Gusmão AL, El-Bachá RS, Ayres MCC (2009) Efeito da suplementação com vitamina E e Selênio sobre o quadro hematológico, enzimas marcadoras de lesão muscular e índice de peroxidação de biomoléculas em equinos submetidos à atividade de salto. Ciênc Anim Bras 10(3):790–801

El-Deeb WM, El-Bahr SM (2010) Investigations of selected biochemical indicators of equine rhabdomyolysis in Arabian horses: pro inflammatory cytokines and oxidative stress markers. Vet Res Commun 34(8):677–89

Etchichury M (2004) Efeitos da suplementação parenteral com selênio e vitamin E nos valores hemáticos e séricos em cavalos de enduro. Dissertação (mestrado)—Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, p85

Fernandes WR, Larsson MHMA (2000) Alterações nas concentrações séricas de glicose, sódio, potássio, uréia e creatinina, em eqüinos submetidos a provas de enduro de 30 km com velocidade controlada. Ciênc Rural 30(3):393–398, Santa Maria

Ferraz GC, D'Angelis FHF, Teixeira-Neto AR, Freitas EVV, Lacerda-Neto JC, Queiroz-Neto A (2008) Blood lactate reflects glucose responses in horses submitted to incremental exercise test. Arq Bras Med Vet Zootec 60(1):256–259

Fonseca LA, Girardi FM, Coelho CS, Barioni G, Rangel VB, Gonçalves RC (2011) Influence of chromium supplementation on energy metabolism in horses used in policing activity. Arq Bras Med Vet Zootec 63(5):1175–1180

Frank N (2005) Insulin resistance in horses. In: Annual Convention of the American Association of Equine Practitioners, vol 52. American Association of Equine Practitioners, Lexington, pp 1–11

Gomide LMW, Martins CB, Orozco CAG, Sampaio RCL, Belli T, Baldissera V, Neto JCL (2006) Concentrações sanguíneas de lactato em equinos durante a prova de fundo do concurso completo de equitação. Ciênc Rural 36(2):509–513

Gontijo LD, Cassou F, Junior PVM, Alves GES, Bringel B, Ribeiro RM, Lago LA, Faleiros RR (2014) Bem-estar em equinos de policiamento em Curitiba/PR: indicadores clínicos, etológicos e ritmo circadiano do cortisol. Ciênc Rural 44(7):1272–1276

Irvine CHG, Alexander SL (1994) Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. Domest Anim Endocrinol 11(2):227–238

Ji LL (1999) Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 222:283–292

Leal BB, Faleiros RR, Alves GES, Douglas RH, Bringel BA, Lago LA, De Marval CA, Haddad JPA (2007) Alterações no ritmo de cortisol em equinos de cavalaria militar relacionadas como maior ocorrência de cólica. In: III Simpósio Internacional do Cavalo Atleta, Belo Horizonte, Anais do III SIMCAV. FUNDEP, Belo Horizonte

Lessa DAB (2003) Doença Inflamatória das Vias Aéreas (DIVA) em equinos de policiamento na Cidade do Rio de Janeiro, RJ: estudo clínico e da atividade macrofágica alveolar. Tese (doutorado). Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. 102 f.

Lewis LD (2000) Nutrição Clínica Equina: Alimentação e Cuidados. Editora Rocca, São Paulo, 710 p

Linden A et al (1990) Relationship of plasma lactate production to cortisol release following completion of different types of sporting events in horses. Vet Res Commun 20:371–379

Lindner A (2000) Use of blood biochemistry for positive performance diagnosis of sports horses in practice. Rev Med Vet 151(7):611–618

Marc M, Parvizi N, Ellendorff F, Kallweit E, Elsaesser F (2000) Plasma cortisol and ACTH concentrations in the warm blood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status. J Anim Sci 78:1936–1946, Champaign

Matteri RL, Carol JA, Dyer CJ (2000) Neuroendocrine responses to stress. In: Moberg GP, Meneh JA (eds) The biology of animal stress. CABI Publishing, Oxon, pp 43–76

McGowan C (2008) Clinical pathology in the racing horse: the role of clinical pathology in assessing fitness and performance in the racehorse. Vet Clin N Am Equine Pract 24:405–421

McKeever KH (2002) The endocrine system and the challenge of exercise. Vet Clin N Am Equine Pract 18:321–353

Moreira DO, Leme FOP, Marques MM, Leão NF, Viana WS, Faleiros RR, Alves GES (2015) Concentrações de proteínas totais, glicose, cálcio, fósforo, lactato, ureia e creatinina em equinos de cavalaria militar antes e após trabalho de patrulhamento urbano. Cienc Anim Bras 16(1):73–80

National Research Council (2007) Nutrient Requirements of Horses. Washington DC: National Academy, p360

Nogueira GP et al (2002) Serum cortisol, lactate and creatinine concentrations in Thoroughbred fillies of different ages and states of training. Braz J Vet Res Anim Sci 39(1):54–57, São Paulo

Pagan JD, Karnezos P, Kennedy MA, Currier T, Hoekstra KE (1999) Effect of selenium source on selenium digestibility and retention in exercised thoroughbreds. Proceedings of 16th Equine Nutrition and Physiology Society pp. 1–4

Powers SK, Ji LL, Leewenburgh C (1999) Exercise training induced alterations in skeletal muscle antioxidant capacity: a brief review. Med Sci Sports Exerc 31(7):987–997

Radostits OM, Gay CC, Hinchcliff KW et al (2007) Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th edn. Elsevier, Philadelphia, p 2156

Ralston SL (2002) Insulin and glucose regulation. Vet Clin N Am Equine Pract 18:295–304

Ribeiro CR, Martins EAN, Ribas JAS, Germinaro A (2004) Avaliação de constituintes séricos em eqüinos e muares submetidos à prova de resistência de 76 km, no Pantanal do Mato Grosso, Brasil. Ciênc Rural 34:1081–1086

Sen CK, Packer L (2000) Thiol homeostasis and supplements in physical exercise. Am J Clin Nutr 72(2):653S–669S

Silveira VF (2005) Malondialdeído, vitamina E, cortisol, hemograma e enzimas musculares em equinos da raça Árabe submetidos ao exercício em esteira de alta velocidade. Dissertação (mestrado), Faculdade de Medicina Veterinária e Zootecnia de Botucatu, Universidade Estadual Paulista, 92 f

Takanami Y, Iwane H, Kawai Y, Shimomitsu T (2000) Vitamin E supplementation and endurance exercise. Are there benefits? Sports Med 9(2):73–83

Teixeira PP, Meirinhos MLG, Pádua JT, Vieira D (2008) Variações cíclicas do cortisol, triiodotironina (t3) e tiroxina (t4) no periparto de éguas da raça quarto de milha. Ciênc Anim Bras 9(2):263–271

Teixeira-Neto AR, Ferraz GC, D'Angelis FHF, Lacerda-Neto JC, Queiroz-Neto A (2007) Exercise intensity, but not electrolyte reposition, alters plasmatic cortisol and glucose levels of horses submitted to 30 and 60 km distance endurance rides. Ciênc Rural 37(3): 740–743, Santa Maria

Vázquez MR, El-Bachá RS, Ordás CA, Ribeiro EB, Vicente JGV, Rodrigues LEA (2006) Dieta afro-bahiana, estrés oxidativo y ejercício físico. Revista de Nutrição 19(6):673–683, Campinas

Williams CA, Carlucci SA (2006) Oral vitamin E supplementation on oxidative stress and antioxidant status in intensely exercised horses. Equine Vet J Suppl 36:617–621, London