http://dx.doi.org/10.1590/1678-4162-12821

Arq. Bras. Med. Vet. Zootec., v.75, n.4, p.554-560, 2023

Cardiorespiratory/sedative effects of a peptide identified in crotalic venom compared to acepromazine and xylazine in horses

[*Efeitos cardiorrespiratórios/sedativos de um peptídeo identificado no veneno crotálico comparado à acepromazina e à xilazina em equinos*]

C. J. X. *Abimussi*[1], M. D. *Eneas*[2], B. P. *Floriano*[3], G. *Picolo*[4]

[1]Faculdade de Medicina, Universidade Federal de Juiz de Fora, UFJF, Juiz de Fora, MG, Brasil
[2]Veterinarian, São Paulo, Brasil
[3]Universidade Federal de Santa Maria, UFSM, Rio Grande do Sul, Brasil.
[4]Instituto Butantã, São Paulo, Brasil

ABSTRACT

The purpose of this study was to investigate whether intravenous crotalphine produces significant sedation, as well as physiological changes, in healthy standing horses. Six mares, aged 8 years and weighing 415kg underwent three different treatments in a crossover design: TA (acepromazine: 50µg.kg^{-1}), TC (crotalphine: 0.01µg.kg^{-1}) and TX (xylazine: 1000µg.kg^{-1}), intravenously. At various time points over 60 minutes, physiologic variables were recorded: heart rate, respiratory rate, and rectal temperature. The head height from the ground (HHG) was evaluated in centimeters. Data were analyzed using ANOVA followed by Dunnett's test or Friedman followed by Dunn's test, under 5% significance. Heart rate decreased significantly at M_5 and M_{10} compared with M_b in TX (28±7, 26±6 and 40±8 beats/minute^{-1}, respectively; p=0.0004). Respiratory rate and rectal temperature did not differ among groups or time points. The HHG significantly decreased in all groups compared with Mb at various time points (p<0.0001). In conclusion, crotalphine did not produce reliable and durable sedation in healthy standing mares and did not influence cardiorespiratory variables in a clinically relevant manner.

Keywords: muscle relaxation activity, horses, sedation

RESUMO

O objetivo deste estudo foi investigar se a administração de crotalfina intravenosa produz sedação significativa e alterações fisiológicas em equinos saudáveis. Seis éguas, idade média de oito anos e peso médio de 415kg, foram submetidas a três tratamentos distintos: TA (acepromazina: 50µg/kg), TC (crotalfina: 0,01µg/kg) e TX xilazina: 1000µg/kg), por via intravenosa. Em vários momentos, ao de longo de 60 minutos, as variáveis fisiológicas registradas foram frequência cardíaca, frequência respiratória e temperatura retal. A altura de cabeça ao solo (ACS) foi avaliada em centímetros. Os dados foram analisados pela ANOVA, seguida pelo teste de Dunnett ou de Friedman e, depois, pelo teste de Dunn, sob 5% de significância. A frequência cardíaca diminuiu significativamente em M5 e M10 em comparação com Mb em TX (28±7, 26±6 e 40±8 bpm, respectivamente; P=0,0004). A frequência respiratória e a temperatura retal não diferiram entre os grupos ou os pontos de tempo. O HHG diminuiu significativamente em todos os grupos em comparação com Mb em vários momentos (P <0,0001). Em conclusão, a crotalfina não produziu sedação confiável e durável em éguas saudáveis e não influenciou as variáveis cardiorrespiratórias de maneira clinicamente relevante.

Palavras-chave: atividade de relaxamento muscular, cavalos, sedação

Corresponding author: caio.abimussi@ufjf.br
Submitted: July 1, 2022. Accepted: February 28, 2023.

INTRODUCTION

In the routine of veterinary practice in large animals, some drugs have a significant role, such as tranquilizers and sedatives, since the use of these substances as components of an anesthetic protocol provides advantages in the approach and management of the patient.

Among the drugs used, acepromazine is a sedative that promotes muscle relaxation, free of analgesic effects (Rankin, 2015), with vasodilating and hypotensive properties (Steffey *et al*., 1985), however, it allows a compensatory response with an increase in cardiac rate (Muir *et al*., 1979; Coulter *et al*., 1981).

Another widely used drug is xylazine, which has sedative, analgesic, and muscle relaxant effects. As it belongs to the class of α-2 adrenergic receptor agonists and these receptors are distributed throughout the body, vasoconstriction (transitory) followed by bradycardia and hypotension are observed as side effects (Biaggioni and Robertson, 2018).

There are reports by Vital Brazil, between 1930 and 1940, suggesting the presence of an analgesic factor in rattlesnake venom, since those caused by crotalic accidents did not report severe pain, but paresthesia (Souza e Silva *et al*., 1996; Picolo *et al.*, 2003). The analgesic compound present in crotalic venom was isolated, synthesized, and then named "crotalphine". Crotalphine is a 14-amino acid peptide (EFSPENCQGESQPC) containing a disulfide bridge and a pyroglutamic acid, synthesized from the sequence of the analgesic compound purified from the snake venom *Crotalus durissus terrificus* (Konno *et al*., 2008). Crotalphine can induce potent and long-lasting analgesic effects in acute and chronic pain models (including cancer pain and neuropathic pain), an effect estimated in rodents (Gutierrez *et al*., 2008; Konno *et al*., 2008). The action mechanism of this effect involves the specific peripheral activation of CB2 receptors, which, once activated, release endogenous peptides, particularly dynorphin A, which is the endogenous agonist of kappa receptors, which causes antinociception by action of the receptors (Machado, 2014).

Some opioids drugs can cause excitement in horses due to their agonistic action at the mu (μ) receptor. For this, the association of sedatives with these opioid agents is used in veterinary anesthesiology. However, kappa (κ) receptor agonies induce, in addition to analgesia, a considerable degree of sedation and, particularly in horses, are used as a mildly acting sedative (Taylor and Clarke, 2009). Notwithstanding the known analgesic effects of crotalphine, to date there are no prospective studies addressing the sedative effects of this peptide in domestic animals.

Therefore, the purpose of this study was to investigate whether intravenous crotalphine produces significant sedation, as well as physiological changes, in healthy standing horses. It is not an objective of this study to assess the analgesic effects of crotalphine in horses.

MATERIALS AND METHODS

This study was carried out at the "Roque Quagliato" Veterinary Hospital of the University Centre of Ourinhos - UNIFIO, after approval by the local Ethics Committee under protocol number 002/2016.

Crotalphine dose extrapolation. The dosage of Crotalphine for horses ($0.01\mu g.kg.^{-1}$) was established through allometric extrapolation, (Pachaly, 2006) from the dose performed in rats of approximately 200g, (0.048 to $6\mu g.kg^{-1}$) being the equine the target animal and rat the known animal (Brigatte *et al*., 2013). We use the lowest dosage due to unknown effects. From the metabolic weight, basal and specific metabolic rate of known and target animals, it was possible to identify the dosage of crotalphine. The final dosage of this allometry was carried out based on the information on the doses used and which did not cause significant side effects in studies already carried out.

Crotalphine solution. From the dosage established by the allometric method, the initial solution in lyophilized form at a temperature of -20°C was diluted in a laminar flow hood. For this dilution, the handler was dressed in sterile gloves, cap, and mask, in order to avoid contamination during the procedure. The vial with the solution containing 1mg (lyophilized)

was diluted in 1mL of sterile water. After, the final dilution remained 1:10 and maintaining the final concentration of 100µg.ml^{-1}. These 10mL were divided into 10 aliquots in Eppendorf's each being 1 ml. All aliquots were kept isolated in a freezer at -20°C and the temperature checked every 12 hours every day.

Animals. Six female horses of various breeds, aged between 3 and 15 years (average 8 years), average weight of 415kg, belonging to the Experimental Farm of University Centre of Ourinhos - UNIFIO were used in this study. Only animals that were considered healthy according to a physical evaluation that included heart rate (HR), respiratory rate (RR), rectal temperature and oral mucosa color, and complete cell count were included. Exclusion criteria were any hematological alterations, clinical alterations, patients undergoing treatment in progress or pregnant females. The animals were randomly subjected to three treatments, by crossover and blind, respecting an interval between them of seven days, as described below:

- TA (acepromazine treatment) - 50µg.kg^{-1} - IV
- CT (crotalphine treatment) - 0.01µg.kg^{-1} - IV
- TX (xylazine treatment) - dose1000 µg.kg^{-1} - IV

Parameters evaluated. Heart rate (HR) in beats per minute (bpm), respiratory rate (RR) in breaths per minute (mpm), rectal temperature (RT) in degrees Celsius (^{o}C), head height from the ground (HHG) were evaluated in centimeters (cm) and ataxia. For this purpose, the moments were considered, where the numbers reflect the minutes from the time of treatment:

- Baseline moment (M_b): immediately before treatment
- Time 1 (M_1): one minute after treatment
- Time 5 (M_5): five minutes after treatment
- Time 10 (M_{10}): ten minutes after treatment
- Time 15 (M_{15}): fifteen minutes after treatment
- Time 30 (M_{30}): thirty minutes after treatment
- Time 60 (M_{60}): sixty minutes after treatment

Head heigh from the ground was assessed using a measuring tape fixed to the restraint trunk, from the animal's lower lip. Ataxia and sedation score were performed using a table with scores (Table 1).

Table 1. Descriptive numerical scale representation for measuring the degrees of ataxia (Guirro *et al*, 2016)

Ataxia Degree	Description
0	Stability
1	Reduction os stability with some lateral movement
2	More intense lateral movement, trend to inclination
3	Pelvic support in the stock, members crossed, sudden and frequent flexion of carpal joints

Data were submitted to Shapiro-Wilk's normality test. Variables that showed normal distribution were submitted to repeated measures analysis of variance and Dunnett's test. Variables that did not show normal distribution were submitted to Friedman's test followed by Dunn's test. The incidence of sweating, lip ptosis and urination were investigated using chi-square test. All differences were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

In TX, the HR values in M_5 and M_{10} were respectively decreased to 28±7 and 26±6 beats/minute^{-1} in relation to M_b (40±8 beats/minute^{-1}). This can be explained by the fact that xylazine is an alpha-2 adrenergic receptor agonist drug, acting to inhibit the release of noradrenaline in the synaptic cleft, with promotes negative chronotropism due to the increase in peripheral vascular resistance as described by Valverde (2010). Muir and Mason

(1993), when evaluating the effect of acepromazine on HR, observed a significant increase as in this study, corroborating the findings of Muir *et al*. (1979); Coulter *et al*. (1981) who described an increase in HR that was compensatory to the decrease in systemic vascular resistance and blood pressure.

To measure the head heigh from the ground (HHG), the authors used a measuring tape attached to a physical restraint trunk for large animals, similar to the method used by Queiroz Neto *et al*. (2001); Christovão *et al*. (2006); Carregaro *et al*. (2007). Thus, it can be observed that HHG, in TA, decreased to 100±23cm, 102±13cm and 92±2- cm in M_{10}, M_{30} and M_{60} respectively when compared to baseline (127±8cm). In the treatment of crotalphine, HHG decreased to 109±7 cm in M_{10} compared to baseline (127±7cm), and in TX, compared to baseline (126±3cm), it decreased to 53±42cm, 52±37cm and 58±26cm in M_5, M_{10} and M_{15}, respectively (Table 2).

Table 2. Heart rate (HR), respiratory rate (RR), rectal temperature (RT) and head height from the ground (HHG) of six mares at baseline (M_b) and following intravenous administration of acepromazine (TA), crotalphine (TC) or xylazine (TX), over 60 minutes (M_1 to M_{60})

Variable	Treatment	Time points						
		MB	M1	M5	M10	M15	M30	M60
HR (beats/ minute^{-1})	TA	43±12	39±10	38±7	43±8	41±8	40±8	37±5
	TC	39±17	38±12	40±9	40±7	39±6	35±5	37±11
	TX	40±8	29±8	28±7*	26±6*	28±7	28±4	34±7
RR (breaths/ minute^{-1})	TA	21±2	20±6	17±5	16±4	15±3	16±5	15±4
	TC	22±11	21±7	25±10	23±11	20±6	21±7	24±8
	TX	23±4	19±7	17±3	15±5	14±6	14±3	17±4
RT (°C)	TA	37.1±0.3	37.2±0.2	36.8±0.4	36.7±0.6	36.6±0.5	36.5±0.6	36.5±0.8
	TC	37.1±0.6	37.2±0.5	37.3±0.6	37.3±0.5	37.3±0.6	37.4±0.6	37.4±0.4
	TX	37.3±0.3	37.6±0.3	37.4±0.4	37.4±0.4	37.5±0.4	37.1±0.6	37.1±0.7
HHG (cm)	TA	127±8	113 ± 16	110 ± 9	100 ± 23*	103 ± 16	102±13*	92±20*
	TC	127±7	114 ± 7	111±7	109±7*	108 ± 9	110±10	118±11
	TX	126±3	86±43	53±42*	52±37*	58±26*	66±33	108±9

*Significantly different from MB within the same groups according to Dunnett or Dunn test ($p<0.05$).

Short (1998) describes that the tranquilizing and sedative effects of acepromazine were more pronounced between 15 and 20 minutes after the administration of doses between 0.02 and 0.06mg.kg^{-1} (IV). In the present study, a maximum measurable effect was observed between 10 and 60 minutes on the mean distance from the head to the ground, after application of acepromazine at a dose of 0.05mg.kg^{-1}. It is already known that alpha-2 agonists cause more reliable sedation than phenothiazines, in addition to being analgesics, which does not occur with acepromazine (Muir and Hubbell, 1991), a fact that can be observed in the present study, corroborating the reports by Clarke *et al*. (1991) and England *et al*. (1992).

No excitation was observed in TA and TC. Castro (1981), reports that 0.3mg.kg^{-1} (IV) of acepromazine caused mild or severe excitation in 11 of 20 animals tested, which did not occur in the present study, although the authors used a lower dose. (0.05mg.kg^{-1}). Christovão *et al*. (2006) and Roscoe (2007) report that calm

horses can mask the effect of the medications administered. In the present study, half of the mares used were calm, which could fit into these animals mentioned by the authors, which disguise the effect of the drug in comparison with M_b.

In addition to the cardiorespiratory parameters and the others already mentioned, other signs were observed, such as: exchange of support, urination, defecation, yawning, flatulence, and sweating. The animals' mean exchange of support during the evaluation period was 17.3 in TX, 25.5 in TA and 18.8 in TC.

Sweating in the TX was negative in four animals, whereas in the TA and TC sweating was negative in all of the animals. There was no relationship between the presence of sweating and the treatments studied (p=0.1054). Elfenbein *et al*. (2009) and Steffey *et al*. (2000) mentioned that, as occurred in this study, sweating in animals that received xylazine was present.

The occurrence of lip and eyelid ptosis together with drowsiness may indicate mild sedation in horses (Castro and Eisenach 1989; Rossi *et al*., 2003). Lip ptosis was observed in five animals in the TX. In the TA, it was obtained in three animals. On TC only one animal had this event and the relationship between treatments and lip ptosis was not significant (p=0.0695). Muir and Hubbell (1991); Wilson *et al*. (2002) reported the occurrence of lip ptosis with the use of acepromazine and xylazine in horses, as happened in the present study in animals using TA and TX.

In TX, five animals urinated, while in the other treatments this was not observed (p=0.0010). The occurrence of urination after the application of alpha-2 agonist drugs has also been described by other authors in other species, as observed by Ribeiro *et al*. (2012) in cattle that received intravenously xylazine or detomidine and by Thurmon *et al*. (1984); Trim and Hanson (1986) on mares and ponies, respectively. Such an event can be explained by the effect of xylazine on one or more water retention mechanisms (inhibition of antidiuretic hormone synthesis or excretion and/or its actions on distal renal tubules (Thurmon *et al*., 1978).

Only at TA, three animals had flatulence during the evaluation period after administration of acepromazine (p=0.0273). At TC, all the animals defecated after the application of crotalphine (p=0.0036) during the evaluation period and TX and TA only one animal defecated. Guirro (2008) reports that, after the administration of crotalphine, 83% of the animals in their study defecated, corroborating the findings in the present study, however, Roger *et al*. (1994) mention that kappa receptor agonist opioids reduce defecation.

Limitations of the study were the low number of animals that participated in the project and its management, in which there should have been an adaptation period, since none of the animals was used to staying for an hour in the containment trunk after going through a period of fasting, causing half the horses to remain restless during the evaluation period.

CONCLUSION

Crotalphine did not produce reliable and durable sedation in healthy standing mares and did not influence cardiorespiratory variables in a clinically relevant manner. Defecation suggests increased gastrointestinal motility, but more studies need to be carried out to investigate this effect.

ACKNOWLEDGEMENT

We acknowledge the University Centre of Ourinhos (UNIFIO) for allowing the study to be carried out in their facilities and the Butantan Institute to provide the peptide.

REFERENCES

BIAGGIONI, I.; ROBERTSON, D. Adrenoceptor agonists & sympathomimetic drugs. In: KATZUNG, B.G. (Ed.). *Basic & clinical pharmacology*. New York: Lange Medical Books/McGraw Hill, 2018. p.137-155.

BRIGATTE, P.; KATSUHIRO, K.; GUTIERRES, V.P. *et al*. Peripheral kappa and delta opioid receptors are involved in the antinociceptive effect of crotalphine in a rat model of cancer pain. *Pharmacol. Biochem. Behav*., v.109, p.1-7, 2013.

BRUST, T.F.; MORGENWECK, J.; KIM, S. *et al*. Biased agonists of the kappa opioid receptor suppress pain and itch without causing sedation or dysphoria. *Sci. Signal*., v.9, p.1-11, 2016.

CARREGARO, A.B.; LUNA, S.P.L.; MATAQUEIRO, M.I. *et al*. Effects of buprenorphine on nociception and spontaneous locomotor activity in horses. *Am. J. Vet. Res.*, v.68, p.246-250, 2007.

CASTRO, E.A. *Emprego do cloridrato de ketamina associado à acepromazina e éter gliceril guaiacol para a indução da anestesia geral pelo halotano em equinos*. 1981. 49f. Dissertação (Mestrado em Medicina Veterinária) - Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

CASTRO, M.I.; EISENACH, J.C. Pharmacokinetics and dynamics of intravenous, intrathecal, and epidural clonidine in sheep. *Anesthesiology*, v.71, p.418-425, 1989.

CHRISTOVÃO, F.G.; ZAMUR, G.; MATAQUEIRO, M.I. *et al*. Sedative and antinociceptive effects of romifidine and xylazine in Thoroughbred mares. *Arq. Bras. Med. Vet. Zootec*., v.58, p.1030-1036, 2006.

CLARKE, K.W.; ENGLAND, G.C.W.; GOOSSENS, L. Sedative and cardiovascular effects of romifidine, alone and in combination with butorphanol, in the horse. *J. Vet. Anaesth*., v.18, p.25-29, 1991.

COULTER, D.B.; WHELAN, S.C.; WILSON, R.C. *et al*. Determination of blood pressure by indirect methods in dogs given acetylpromazine maleate. *Cornell Vet*., v.71, p.75-84, 1981

ELFENBEIN, J.R.; SANCHEZ, L.C.; ROBERTSON, S.A. *et al*. Effect of detomidine on visceral and somatic nociception and duodenal motility in conscious adult horses. *Vet. Anaesth. Analg.*, v.36, p.162-172, 2009.

ENGLAND, G.C.; CLARKE, K.W.; GOOSSENS, L.A. Comparison of the sedative effects of three α_2-adrenoceptor agonists (romifidine, detomidine and xylazine) in the horse. *J. Vet. Pharmacol. Ther*., v.15, p.194-201, 1992.

GUIRRO, E.C.B.P. *Efeitos comportamental, clínico e antinociceptivo da crotalfina em equinos:* comparação com morfina, U50-488h e fenilbutazona. 2008. 80f Tese (Doutorado em Cirurgia Veterinária) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP.

GUIRRO, E.C.B.P.; PEROTTA, J.H.; PAULA, M.; CURY, Y.; VALADÃO, C.A.A. Clinical, behavioral and antinociceptive effects of crotalphine in horses. *Cienc. Rural*, v.46, p.694-699, 2016.

GUTIERREZ, V.P.; KONNO, K.; CHACUR, M. *et al*. Crotalphine induces potent antinociception in neuropathic pain by acting at peripheral opioid receptors. *Eur. J. Pharmacol.*, v.594, p.84-92, 2008.

KONNO, K.; PICOLO, G.; GUTIERREZ, V.P. *et al*. Crotalphine, a novel potent analgesic peptide from the venom of the South American rattlesnake *Crotalus duress's terrificus*. *Peptides*, v.29, p.1293-1304, 2008.

MACHADO, F.C. Peripheral interactions between cannabinoid and opioid systems contribute to the antinociceptive effect of crotalphine. *Br. J. Pharmacol*., v.171, p.961-972, 2014.

MUIR, W.W.; MASON, D.E. Effects of diazepam, acepromazine, detomidine, xylazine on thiamilal anesthesia in horses. *J. Am. Vet. Med. Assoc.*, v.203, p.1031-1038, 1993.

MUIR, W.W.; HUBBELL, J.A.E. Equine anesthesia. In: _____. *Standing chemical restraint in horses*: tranquilizers, sedatives, and analgesics. St Louls: Mosby-Yer Book, 1991. cap.11, p.247-280.

MUIR, W.W.; SKARDA, R.T.; SHEERAN, W. Hemodynamic and respiratory effects of a xylazine-acetylpromazine drug combination in horses. *Am. J. Vet. Res.*, v.40, p.1518-1522, 1979.

PACHALY, J.R. Terapêutica por extrapolação alométrica. In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L. (Eds.). *Tratado de animais selvagens* - medicina veterinária. São Paulo: Roca, 2006. p.1215-1223.

PICOLO, G.; CASSOLA, A.C.; CURY, Y. Activation of peripheral ATP-sensitive K+ channels mediates the antinociceptive effect of Crotalus durissus terrificus snake venom. *Eur. J. Pharmacol.*, v.469, p.57-64, 2003.

QUEIROZ NETO, A.; ZAMUR, G.; CARREGARO, A.B. *et al*. Effects of caffeine on locomotor activity of horses: determination of the no-effect threshold. *J. Appl. Toxicol*., v.21, p.229-234, 2001.

RANKIN, D.C. Sedative and tranquilizers. In: GRIMM, K.A.; LAMONT, L.A.; TRANQUILLI, W.J.; GREENE, S.A.; ROBERTSON, S.A. (Eds.). *Veterinary anesthesia and analgesia*: Lumb and Jones. 5.ed. IOWA: [Wiley-Blackwell], 2015, p.193-206.

RIBEIRO, G.; DÓRIA, R.G.S.; NUNES, T.C. *et al.* Efeitos da detomidina e xilazina intravenosa sobre as variáveis basais e respostas comportamentais em bovinos. *Arq. Bras. Med. Vet. Zootec.*, v.64, p.1411-1417, 2012.

ROGER, T.; BARDON, T.; RUCKESBUSCH, Y. Comparative effects of mu and kappa opiaite agonists on the cecocolic motility in the pony. *Can. J. Vet. Res.*, v.58, p.163-166, 1994.

ROSCOE, M.P. *Avaliação de seis protocolos de sedação para procedimentos odontológicos em equinos*. 2007. 57f. Dissertação (Mestrado em Ciência Animal) - Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

ROSSI, R.; BUCKER, G.V.; VARELA J.V. Peripheral analgesic actions of peripheal clonidine in cattle. *Vet. Anaesth. Analg.*, v.30, p.63-70, 2003.

SHORT, C.E. Fundamentals of pain perception in animals. *Appl. Anim. Behav. Sci.*, v.59, p.125-133, 1998.

SOUZA E SILVA, M.C.C.; GONÇALVEZ, L.R.C.; MARIANO, M. The venom of South American Ratllesnake inhibits macrophage functions and is endowed with anti-inflammatory properties. *Mediat. Inflamm.*, v.5, p.18-23, 1996.

STEFFEY, E.P.; KELLY, A.B.; FARVER, T.B. *et al*. Cardiovascular and respiratory effects of acetylpromazine and xylazine on halothane anesthetized horses. *J. Vet. Pharmacol. Ther.*, v.8, p.290-302, 1985.

STEFFEY, E.P.; PASCOE, P.J.; WOLINER, M.J. *et al*. Effects of xylazine hydrochloride during isoflurane-induced anesthesia in horses. *Am. J. Vet. Res.*, v.61, p.1225-1231, 2000.

TAYLOR, P.M.; CLARKE, K.W. *Manual de anestesia em equinos*. São Paulo: MedVet, 2009. 334p.

THURMON, J.C.; NELSON, D.R.; HARTSFIELD, S.M. *et al*. Effects of xilazine hydrochloride on urine in cattle. *Aust. Vet. J.*, v.54, p.178-180, 1978.

THURMON, J.C.; STEFFEY, E.P.; ZINKL, J.G. *et al*. Xylazine causes transient dose related hyperglycemia and increased urine volumes in mares. *Am. J. Vet. Res.*, v.45, p.224-227, 1984.

TRIM, C.N.; HANSON, R.R. Effects of xylazine on renal function and plasma glucose in ponies. *Vet. Rec.*, v.118, p.65-67, 1986.

VALVERDE, A. Alpha-2 agonists as pain therapy in horses. *Vet. Clin. North. Am. Equine Pract.*, v. 26, p.515-532, 2010.

WILSON, D.V. BOHART, G.V. EVANS, A.T. *et al.* Retrospective analysis of detomidine infusion for standing chemical restraint in 51 horses. *Vet. Anaesth. Analg.*, v.29, p.54-57, 2002.