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**EFFECT OF LONG DETOMIDINE CONSTANT RATE
INFUSION WITH AND WITHOUT VATINOXAN ON
GASTROINTESTINAL MOTILITY IN HEALTHY HORSES**

PIKKAAJALISE DETOMIDIINI PÜSIINFUSIOONI MÕJU KOOS
VATINOKSAANIGA JA ILMA SELLETA TERVETE HOBUSTE
SOOLEMOTOORIKALE

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<p>Detomidine is an alpha-2-agonist commonly used in horse sedation. It has negative side effects on gastrointestinal motility that can increase the risk of colic. This experimental crossover study evaluated the effects of a peripherally acting alpha-2-antagonist (vatinoxan) on gastrointestinal motility in healthy horses sedated with detomidine. Eight horses received two four-hour infusions of either detomidine hydrochloride (0.01 mg/kg + 0.015 mg/kg/h IV) alone or combined with vatinoxan hydrochloride (0.15 mg/kg + 0.05 mg/kg/h IV). Small plastic spheres were administered with a nasogastric tube before the start of infusion. Borborygmi scores were monitored. The time for the first defecation was marked down. The excretion of the spheres, faecal output, and frequency of defecation were monitored repeatedly after the end of the infusion. Reduction in borborygmi scores was less with the combination of detomidine and vatinoxan than with detomidine ($p < 0.05$). With vatinoxan, the mean cumulative weight of faeces and the cumulative number of faecal piles were higher during the first 8 hours after the end of infusion than with detomidine alone ($p < 0.05$), and the horses defecated sooner ($p = 0.01$). The plastic spheres were excreted faster with detomidine than with the combination ($p < 0.05$). In conclusion, combining vatinoxan with detomidine improved the borborygmi score, and horses passed faeces sooner, more frequently, and in higher quantities.</p> <p>Therefore, vatinoxan may aid gastrointestinal motility in horses treated with detomidine, although this conclusion is not supported by the slower excretion of spheres in the presence of vatinoxan. Thus, further studies are required to resolve these conflicting results.</p>			
Keywords: detomidine, vatinoxan, equine, gastrointestinal effects			

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<p>Õppetool: kliinilise veterinaarmeditsiini õppetool ETIS-e teadusvaldkond ja CERC S-i kood: 3. Terviseuuringud, 3.2. Veterinaarmeditsiin B750 Veterinaarmeditsiin, kirurgia, füsioloogia, patoloogia, kliinilised uuringud Juhendaja(d): Marja Raekallio ja Aleksandr Semjonov Kaitsmiskoht ja -aasta: Tartu 2024</p> <p>Detomidiin on alfa-2-agonist, mida kasutatakse sageli hobuste rahustamiseks. Sellel on negatiivsed kõrvalmõjud seedetrakti liikuvusele, mis võib suurendada koolikute riski. See eksperimentaalne risturing hindas perifeerselt toimiva alfa-2-antagonisti (vatinoksaani) mõju soolemotoorikale tervetel detomidiiniga rahustatud hobustel. Kaheksa hobust said kas ainult detomidiinvesinikkloriidi (0,01 mg/kg + 0,015 mg/kg/h IV) või kombinatsioonis vatinoksaani vesinikkloriidiga (0,15 mg/kg + 0,05 mg/kg/h IV) kaks neljatunnist infusiooni. Enne infusiooni algust manustati väikesed värvilised plastikkerakesed nasogastraalsondiga. Borborygmi skoori jälgiti. Esimese roojamise aeg fikseeriti. Kerakeste eritumist, väljaheite kogust ja roojamise sagedust jälgiti korduvalt pärast infusiooni lõppu. Borborygmi hinde vähenemine oli detomidiini ja vatinoksaani kombinatsiooniga väiksem kui ainult detomidiiniga ($p < 0,05$). Vatinoksaaniga oli keskmine väljaheite kogukaal ja väljaheitekoguste koguarv esimese 8 tunni jooksul pärast infusiooni lõppu suurem kui ainult detomidiiniga ($p < 0,05$) ja hobused roojasid varem ($p = 0,01$). Plastikkerakesed eritusid detomidiiniga kiiremini kui kombinatsiooniga ($p < 0,05$). Kokkuvõttes parandas vatinoksaani kombineerimine detomidiiniga Borborygmi skoori ning hobused eritasid väljaheiteid kiiremini, sagedamini ja suuremates kogustes.</p> <p>Seega võib vatinoksaan aidata seedetrakti liikuvust detomidiiniga ravitud hobustel, kuigi seda järeldust ei toeta kerakeste aeglasem eritumine vatinoksaani juuresolekul. Seetõttu on nende vastuoluliste tulemuste lahendamiseks vajalikud täiendavad uuringud.</p>			
Märksõnad: detomidiini, vatinoksaan, hobune, seedetrakti mõjud			

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ABBREVIATIONS

CNS - Central nervous system

CRI - constant rate infusion

GI - gastrointestinal

IV - intravenous

NE - norepinephrine

INTRODUCTION

Sedation is a crucial aspect of equine veterinary care, particularly during various procedures such as ocular surgeries (Robertson, 2004), dental interventions (Dixon *et al.*, 2005), castration, and wound suturing (Dugdale *et al.*, 2020). A common sedation protocol involves the use of an alpha-2-agonist in combination with other drugs, such as opioids or acepromazine (Dugdale *et al.*, 2020). Among the alpha-2-agonists, detomidine hydrochloride is licensed for use in horses and finds application in sedation, visceral antinociception, and premedication before general anaesthesia (Tapio *et al.*, 2018).

However, the use of alpha-2-agonists, including detomidine, is associated with various side effects, such as a reduction in gastrointestinal (GI) motility (Zullian *et al.*, 2011), cardiovascular impacts (Yamashita *et al.*, 2000), respiratory effects (Nyman *et al.*, 2009), and disruptions in glucose homeostasis (Kritchevsky *et al.*, 2020). The variation in pharmacodynamics and susceptibility across species is linked to the presence and percentage of alpha-2-adrenergic receptor subtypes. Since alpha-2-agonistic drugs lack receptor subtype specificity, their side effects can be generalized (Posner, 2018).

To mitigate the cardiovascular effects of alpha-2-agonists, interventions, such as anticholinergics (e.g., atropine), and alpha-2-antagonists (e.g., yohimbine or atipamezole), have been employed (Dugdale *et al.*, 2020). Despite its effectiveness in preventing alpha-2-agonist-induced bradycardia and other cardiac issues, atropine is not recommended in combination with alpha-2-agonists due to its potential to cause severe hypertension (Pimenta *et al.*, 2011). Atipamezole, a centrally and peripherally acting alpha-2-antagonist, has been indicated for the reversal of alpha-2-agonists, effectively counteracting reductions in heart rate and the frequency of atrioventricular blocks (Raekallio *et al.*, 1990).

The decrease in intestinal motility induced by alpha-2-agonists poses risks such as impaction, postoperative ileus, and post-anaesthetic colic (Zullian *et al.*, 2011; Vainionpää *et al.*, 2013; Tapio *et al.*, 2018; Tapio *et al.*, 2019). While atipamezole has demonstrated efficacy in antagonizing alpha-2-agonist effects on GI motility (Zullian *et al.*, 2011), its central actions also counteract alpha-2-agonist-induced sedation (Raekallio *et al.*, 1990). The relatively poorly studied alpha-2-antagonist vatinoxan which displays poor penetration through the blood-brain barrier is a potential candidate for this purpose.

Despite the early research on vatinoxan dating back to the 1980s, there remains a notable paucity of research on its effects, particularly concerning the GI tract in horses. This paper not only explores vatinoxan but also delves into the effects of other alpha-2-antagonists, such as atipamezole and yohimbine, on various species, reflecting the limited recent research on vatinoxan.

A crossover experimental study involved eight Finn horses to investigate the effects of vatinoxan, an antagonist, on the commonly used sedative detomidine. This clinical trial aimed to assess the possible inhibitory effects of vatinoxan on detomidine-induced GI suppression. During the study, the horses were subjected to a four-hour infusion with either detomidine alone or in combination with vatinoxan. Various parameters including borborygmi scores, faecal weight, frequency of defecation, excretion of plastic spheres, and time of first defecation from the start of infusion and up to 72 hours thereafter were recorded and compared between the two treatment groups.

1. LITERATURE REVIEW

1.1. Alpha-2-adrenergic receptors

Alpha-2-adrenergic receptors situated both pre- and post-synaptically (Dugdale *et al.*, 2020) within the central nervous system (CNS), belong to G-protein receptors (Posner, 2018). Their activation induces the inhibition of adenylyl cyclase activity, subsequently leading to a reduction in intracellular cyclic adenosine monophosphate levels and the release of norepinephrine (NE) from the nerve terminals (McMurphy *et al.*, 2018; Posner, 2018). The sedative effect is orchestrated through the binding of alpha-2-agonists with receptors located supraspinally in the locus coeruleus of the pons (Correa-Sales *et al.*, 1992). The ensuing decrease in NE levels diminishes neurotransmission and arousal, resulting in sedation. Some receptors are also present in the endothelium of blood vessels and platelets (Posner, 2018).

Four distinct subtypes of receptors exist, namely alpha-2-A, alpha-2-B, alpha-2-C, and alpha-2-D receptors (Rowe *et al.*, 2018). Alpha-2-A receptors are situated in the presynaptic adrenergic nerve terminals, the CNS, brainstem, spinal cord, postganglionic sympathetic nervous system neurons, autonomic ganglia, and platelets (Posner, 2018). Activation of these receptors is intricately linked to awareness and arousal. Alpha-2-B receptors are found in vascular smooth muscle, the CNS, and the liver and kidneys, eliciting vasoconstriction upon activation (Posner, 2018; Rowe *et al.*, 2018). Alpha-2-C receptors are primarily located in the CNS (Rowe *et al.*, 2018). Cattle exhibit a higher proportion of alpha-2-D receptors, associated with arousal (McMurphy *et al.*, 2018; Posner, 2018). Notably, alpha-2-A, -B, and -C receptors are implicated in antinociception (Dugdale *et al.*, 2020).

1.2. Alpha-2-adrenergic receptor agonists

Compounds that elicit the activation of alpha-2-adrenergic receptors are classified as alpha-2-adrenergic agonists. These agents possess sedative properties, accompanied by analgesic and muscle-relaxant capabilities. Acting both centrally and peripherally, alpha-2-agonists are characterized by the presence of a benzene ring, with xylazine additionally featuring a thiazine ring, while medetomidine, dexmedetomidine, and detomidine incorporate an imidazole ring. Notably, despite being an imidazole derivative, romifidine lacks an imidazole ring. Xylazine (20 and 100 mg/mL), detomidine (10 mg/mL), and romifidine (10 mg/mL) are licensed for equine use (Dugdale *et al.*, 2020).

According to Dugdale *et al.* (2020), the functional attributes of alpha-2-agonists are dependent on multiple mechanisms: 1) Enhancement with alpha-2 (and alpha-1) receptors, 2) Interaction with imidazole receptors (I1, I2, and I3), 3) Manifestation of local anaesthetic-like effects through modulation of ion channels, and 4) Membrane effects, demonstrating independence from specific receptor/ion channel effects. Numerous pharmaceutical agents within this category do not exclusively function as alpha-2-agonists, thereby exhibiting activation of alternative receptors (Dugdale *et al.*, 2020).

For instance, xylazine, medetomidine, and dexmedetomidine display partial activity in alpha-1 receptors. However, it is noteworthy that medetomidine and dexmedetomidine exhibit heightened affinity for alpha-2 receptors. Romifidine, classified as an imidazole derivate, concurrently activates imidazole receptors (Dugdale *et al.*, 2020). The imidazole-1 receptors are implicated in central blood pressure regulation, contributing to hypotension, while imidazole-2 receptors may be linked to analgesic effects (Posner, 2018).

1.2.1. Pharmacokinetics and pharmacodynamics

Variations in pharmacodynamics and susceptibility across species are closely tied to the distribution and proportion of alpha-2-adrenergic receptor subtypes. Notably, none of the injectable alpha-2-agonistic drugs authorized for animal use exhibit specificity towards receptor subtypes. These alpha-2-agonists possess the ability to penetrate the blood-brain barrier, with their pharmacokinetics subject to species variation, specific drug properties, and dosage (Posner, 2018).

Upon intravenous (IV) administration, these drugs typically manifest their effects within minutes, peaking within five to 15 minutes (Garcia-Villar *et al.*, 1981; Mama *et al.*, 2009; Vainionpää *et al.*, 2013). The equipotent sedative doses for horses are as follows: xylazine at 1 mg/kg, medetomidine at 0.005-0.01 mg/kg, romifidine at 0.04-0.08 mg/kg, and detomidine at 0.02-0.04 mg/kg (England *et al.*, 1992). Among these, studies have demonstrated that xylazine exhibits the shortest duration of action, while detomidine elicits the longest-lasting sedative effect (Hamm *et al.*, 1995; Freeman and England, 2000; Yamashita *et al.*, 2002).

Detomidine, specifically, induces the longest duration of ataxia in horses, with maximal ataxia typically observed five minutes post-administration (England *et al.*, 1992; Hamm *et al.*, 1995). Following rest, IV administration of 0.04 mg/kg detomidine resulted in a median half-life of

26 minutes and a median clearance of 16 mL/min/kg. Post-exercise administration extended the median half-life by 20 minutes, increased median clearance by four minutes, and augmented the volume of distribution from 585 to 1296 mL/kg (Hubbell *et al.*, 2009).

Metabolism primarily occurs in the liver, with excretion via urine, though minimal amounts of unmetabolized drugs may be present. Despite the production of various metabolites, their activity remains minimal. Tolerance to repeated doses may arise due to hepatic enzyme induction, receptor downregulation or desensitization, or central alpha-1-receptor stimulation causing arousal (Dugdale *et al.*, 2020).

Detomidine exhibits a longer elimination half-life compared to medetomidine and xylazine (Garcia-Villar *et al.*, 1981; Salonen *et al.*, 1989; Bettschart-Wolfensberger *et al.*, 1999). Ruminants, notably, demonstrate heightened sensitivity to xylazine, attributed to a higher concentration of alpha-2-D-receptors in the CNS (Posner, 2018).

The peak effect of alpha-2-agonists following intramuscular administration is delayed compared to IV administration, with bioavailability ranging from 17 % to 95 % depending on the species (Garcia-Villar *et al.*, 1981). Moreover, the intramuscular bioavailability of alpha-2-agonists varies among species; for instance, detomidine exhibits 85 % bioavailability in cows and 66 % in horses (Salonen *et al.*, 1989). Despite notable clinical effects lasting up to 7 hours, there appears to be a disconnection between pharmacodynamic effects and plasma levels, attributed to either prolonged tissue binding or active metabolites, as plasma levels remain detectable for only 2 hours (Ranheim *et al.*, 1999; 2000; Posner, 2018).

1.2.2. Central nervous system

By selectively binding to and modulating the activity of alpha-2-adrenoceptors situated within the locus coeruleus in the pons and brainstem, alpha-2-agonists prevent the release of NE, thereby attenuating its arousal-inducing effects and induce a state of sedation (Correa-Sales *et al.*, 1992; Sinclair, 2003). Alpha-2-agonists can produce reliable sedation in most species, and the depth and duration are dose-dependent. Lower dosages induce mild to moderate sedation, while higher doses have demonstrated the capability to induce unconsciousness in most species. Notably, in certain species such as horses, a threshold exists for the depth of sedation; elevating the dose beyond this threshold primarily extends the duration of sedation without causing recumbency (Posner, 2018).

It is relevant to acknowledge that presently available alpha-2-agonistic pharmaceuticals exhibit varying degrees of alpha-1-agonistic activity. This property can result in undesired side effects, including agitation, heightened arousal, and increased vigilance, particularly in the case of non-specific drugs such as xylazine (Sinclair, 2003).

1.2.3. Antinociception

Alpha-2-agonists can be administered via parenteral and neuraxial routes, eliciting profound analgesic effects by activating receptors located in the locus coeruleus and the substantia gelatinosa of the dorsal horn of the spinal cord (Guo *et al.*, 1996; Posner, 2018). In the brain, analgesia is achieved through a reduction in neural conduction, while in the spinal cord, it is mediated by the diminished release of neurotransmitters such as NE and substance P (Posner, 2018).

However, the analgesic efficacy of alpha-2-agonists is not without limitations, such as the occurrence of sedation and ataxia. Notably, achieving visceral analgesia requires a higher plasma concentration compared to the levels required for sedation, with equine studies suggesting analgesic levels to be up to 10 times greater than sedative levels (Elfenbein *et al.*, 2009).

To alleviate sedative and cardiovascular side effects, epidural administration emerges as a viable strategy to deliver potent analgesia (Greene *et al.*, 1995; Aminkov and Pascalev, 1998). However, due to the high lipophilicity of alpha-2-agonists, systemic absorption occurs even with epidural administration, resulting in observable, although slight, sedation (Posner, 2018).

1.2.4. Musculoskeletal effects

Alpha-2-agonists can induce good muscle relaxation, which is why they are frequently used with drugs that lack this ability, such as ketamine (Posner, 2018). Notably, certain species, including canines, may manifest side effects such as tremors, facial twitching, or head bobbing in response to alpha-2-agonist administration (Sinclair, 2003). Moreover, these pharmacological agents have an influence on myometrial tone and contractions, although dosage, species type, and the stage of the reproductive cycle also play a role (Posner, 2018). The circulating levels of oestrogen and progesterone modulate the alpha-2-adrenergic receptors, with oestrogen facilitating up-regulation and progesterone inducing down-

regulation, thereby contributing to variations in myometrial contractility (Re *et al.*, 2002; Posner, 2018).

1.2.5. Side effects

1.2.5.1. Cardiovascular system

Alpha-2-agonists elicit a complex cardiovascular response characterized by two distinct phases (Posner, 2018). Initially, activation of presynaptic alpha-2-A receptors in the CNS leads to reduced NE release and an increase in parasympathetic tone (Posner, 2018; Rowe *et al.*, 2018). This surge in parasympathetic activity dampens the ionotropic, chronotropic, and dromotropic effects on the heart, resulting in peripheral vasodilation. Elevated vagal tone and dromotropic effects frequently manifest as first and second-degree atrioventricular blocks. Meanwhile, activation of alpha-2-B and alpha-1-adrenergic receptors in the peripheral vascular endothelium induces vasoconstriction, counteracting the initial vasodilation driven by decreased NE levels. Consequently, blood pressure rises, triggering reflex bradycardia via baroreceptor activation (Posner, 2018).

In the second phase, diminished activation of peripheral endothelial alpha-2-receptors leads to decreased vascular resistance, thereby lowering blood pressure. Towards the end of this phase, patients often experience hypotension and bradycardia, with heart rate remaining low. Reflex bradycardia not only reduces cardiac output but is also influenced by concurrent reductions in stroke volume, increased afterload, and catecholamine deficiency (Posner, 2018).

While alpha-2-agonists typically induce a biphasic blood pressure response, studies in horses utilizing romifidine, medetomidine, detomidine, and xylazine have reported variability in individual responses (de Vries *et al.*, 2016; Yamashita *et al.*, 2000). Romifidine's alpha-2:alpha-1-adrenoceptor selectivity ratio, suggesting partial alpha-2-agonism, may account for the absence of hypotensive effects observed in some cases (de Vries *et al.*, 2016). Yamashita *et al.* (2000) found no hypotensive response in horses receiving 0.01 mg/kg of medetomidine or 0.01-0.04 mg/kg of detomidine, attributing detomidine's lack of hypotension to increased peripheral vascular resistance. Moreover, detomidine exhibited more pronounced and prolonged cardiovascular effects compared to medetomidine and xylazine in a comparative study across different doses. Even at a lower dose than the suggested equipotent sedative dose, detomidine significantly decreased heart rate (Yamashita *et al.*, 2000).

While the cardiovascular effects of alpha-2-agonists are dose-dependent, there appears to be a ceiling dose beyond which cardiovascular effects do not intensify, but sedation, analgesia, and duration of action deepen (Pypendop and Verstegen, 1998; Kuusela *et al.*, 2000). Yamashita *et al.* (2000) observed that detomidine induced a more persistent second-degree atrioventricular block, particularly at higher doses (0.02 mg/kg and 0.04 mg/kg), suggesting prolonged elevation of peripheral vagal tone and suppression of CNS sympathetic outflow. This extended cardiovascular effect may be attributed to detomidine's longer elimination time (Yamashita *et al.*, 2000).

1.2.5.2. Respiratory system

Alpha-2-agonists have been shown to reduce respiratory rate and minute ventilation in several studies (Kollias-Baker *et al.*, 1993; Posner, 2018; Pypendop and Verstegen, 1999; Sinclair, 2003). Sinclair (2003) linked this respiratory depression to CNS depression. While some alpha-2-agonists may not individually induce significant respiratory effects, their combination with other sedatives can compound depression, potentially leading to hypoxemia or cyanosis (Sinclair, 2003). De Vries *et al.* (2016) proposed that romifidine-induced reduction in respiratory rate is attributable to its sedative properties, which decrease ventilatory responsiveness and respiratory sensitivity. This decline in respiratory function correlated with a significant decrease in the arterial partial pressure of oxygen and an increase in the arterial partial pressure of carbon dioxide, likely due to hypoventilation and perfusion mismatch (de Vries *et al.*, 2016).

Contrary to these findings, Lawless *et al.* (2021) observed that intraoperative respiratory rates remained within the normal range during constant rate infusion (CRI) treatments with either romifidine or detomidine. However, Nyman *et al.* (2009) reported a ventilation-perfusion mismatch in horses sedated with detomidine, leading to hypoxemia, and increased physiological dead space. Although detomidine induced impaired pulmonary gas exchange and arterial oxygenation, its effects on respiratory rate were not observed (Nyman *et al.*, 2009).

Yamashita *et al.* (2000) conducted a study on horses sedated with varying doses of medetomidine, detomidine, or xylazine. They found decreased respiratory rates with all doses of detomidine and higher doses of medetomidine, yet hypoventilation did not occur in any of the horses. Additionally, a reduction in arterial partial pressure of oxygen was noted, attributed

to ventilation-perfusion mismatch caused by decreased cardiac index and lung vessel vasoconstriction (Yamashita *et al.*, 2000).

1.2.5.3. Gastrointestinal tract

Alpha-2-agonists are known to decrease GI motility by reducing intestinal contractions, thereby prolonging intestinal transit time (Zullian *et al.*, 2011; de Vries *et al.*, 2016; Posner, 2018; Elfenbein *et al.*, 2019; Gozalo-Marcilla *et al.*, 2019). It has been observed that the large intestine, particularly in horses and dogs, exhibits greater sensitivity to the effects of alpha-2-agonists compared to the small intestine (Maugeri *et al.*, 1994; Sasaki *et al.*, 2000). Maugeri *et al.* (1994) additionally noted a biphasic effect in the canine colon, where alpha-2-agonists initially increased muscle tone, coinciding with peak sedation, followed by a subsequent inhibition of colonic motility.

In a study by Zullian *et al.* (2011), the effects of alpha-2-agonists on intestinal motility were investigated *in vitro* in horses by electrically inducing contractions. All agonists tested (xylazine, detomidine, and medetomidine) demonstrated inhibitory effects on jejunal tissue contractions, with medetomidine exhibiting the highest potency. Furthermore, Posner (2018) reported that alpha-2-agonists decrease gastric acid secretion.

Gozalo-Marcilla *et al.* (2019) conducted a study involving horses receiving five different treatments, consisting of bolus administration followed by a two-hour CRI. These treatments included saline, low-dose, and high-dose detomidine, and low-dose and high-dose detomidine with methadone. Intestinal motility was assessed by auscultating each abdominal quadrant and assigning a motility score ranging from zero to five. It was found that all detomidine-containing treatments resulted in reduced intestinal motility, with the low-dose detomidine treatment decreasing motility for up to 135 minutes and the high-dose detomidine treatment prolonging the decrease for up to 210 minutes (Gozalo-Marcilla *et al.*, 2019).

Lawless *et al.* (2021) investigated the effects of detomidine and romifidine on faecal production in healthy mares undergoing laparoscopic ovariectomy. Following an initial bolus, a CRI was employed to maintain anaesthesia. Faecal output was monitored for the first 12-24 hours preoperatively and 12 hours post-surgery. Both detomidine and romifidine administrations were associated with a reduction in faecal output, as reported by Lawless *et al.* (2021).

1.2.5.4. Renal system

Alpha-2-agonists on renal function and urine production. Firstly, they decrease the production or secretion of antidiuretic hormone from the pituitary gland (Humphreys *et al.*, 1975). This action is coupled with the inhibition of antidiuretic hormone effects on the collecting tubules, leading to increased sodium excretion in the kidneys, consequently promoting water excretion and natriuresis (Smyth *et al.*, 1985; Gellai and Edwards, 1988).

Moreover, alpha-2-agonists directly activate renal alpha-2-adrenergic receptors. This activation, along with an initial hypertensive response, leads to decreased renin levels and increased diuresis (Smyth *et al.*, 1987). Furthermore, they have been observed to decrease micturition pressure, volume, and bladder capacity through spinal and peripheral mechanisms (Ishizuka *et al.*, 1996). Consequently, these actions collectively result in increased production of diluted urine and frequency of urination (Smyth *et al.*, 1987; Ishizuka *et al.*, 1996).

Additionally, alpha-2-antagonists stimulate renal alpha-2 receptors, which reduces renin secretion. This reduction subsequently decreases angiotensin II release, leading to an increase in glomerular filtration rate, natriuresis, and diuresis. Furthermore, reduced cardiac output may also influence the glomerular filtration rate. Finally, the decrease in angiotensin II production reduces aldosterone secretion, further promoting diuresis and natriuresis (Dugdale *et al.*, 2020).

1.2.5.5. Insulin and glucose

Alpha-2-agonists exert varying effects on glucose homeostasis, contingent upon factors such as the specific drug administered, dosage, and the species under consideration (Posner, 2018). Notably, these agonists initially provoke a reduction in insulin secretion from pancreatic beta-cells, succeeded by a subsequent elevation in plasma insulin concentrations (Kritchevsky *et al.*, 2020; Box *et al.*, 2021). Additionally, a concomitant decrease in glucagon release has been documented (Box *et al.*, 2021).

This diminution in insulin levels precipitates heightened glycogenolysis and gluconeogenesis in the liver. Consequently, coupled with a reduction in glucose uptake by peripheral tissues, this cascade of events culminates in the manifestation of hyperglycaemia (Dugdale *et al.*, 2020). Typically, hyperglycaemia does not surpass the renal threshold for glucose (~10 mmol/L), and glucosuria is an infrequent occurrence, barring specific instances in cattle and horses (Posner, 2018; Dugdale *et al.*, 2020).

The initial decline in insulin levels induces an elevation in blood glucose levels, resulting in hyperglycaemia. Xylazine, for instance, has been reported to cause an initial decrease in insulin levels following a marked increase. Detomidine administration leads to a decrease in insulin levels without subsequent increase above baseline (Kritchevsky *et al.*, 2020).

1.2.6. Detomidine

Detomidine is primarily used in equine medicine, although its efficacy extends to other species as well (Rankin, 2015). While it reliably induces chemical restraint for standing sedation in horses, more invasive procedures, such as abdominal surgeries and sinus operations, necessitate supplementary analgesics and sedatives (Wilson *et al.*, 2002).

Gozalo-Marcilla *et al.* (2019) conducted a study investigating the sedative and antinociceptive effects of detomidine through CRIs at various dosages. They found that a high dose of detomidine (0.005 mg/kg), as well as a high dose combined with methadone (0.005 mg/kg detomidine + 0.2 mg/kg methadone followed by two concurrent CRIs of detomidine 0.0125 mg/kg/h + methadone 0.05 mg/kg/h), effectively provided sedation and antinociception in horses. Low doses (0.0025 mg/kg) of detomidine alone lacked significant antinociceptive effects, but when combined with methadone (0.2 mg/kg), similar antinociception to a high dose of detomidine alone was observed (Gozalo-Marcilla *et al.*, 2019).

Detomidine typically induces peak sedation within approximately 5 minutes, lasting for about an hour (Hubbell *et al.*, 2004). In cases of GI pain in horses, detomidine's effects can persist for several hours, especially with doses exceeding 0.02 mg/kg (Rankin, 2015). For equine visceral pain, Malone *et al.* (2002) recommend doses ranging from 0.005 to 0.04 mg/kg IV. An IV dose of 0.01 mg/kg can lead to a decrease in heart rate within 2 minutes of administration and may precipitate a sinoatrial or second-degree atrioventricular block (Buhl *et al.*, 2007; Yamashita *et al.*, 2000).

While detomidine administration alone does not significantly affect respiration, it can lead to an increase in the partial pressure of carbon dioxide and a decrease in the partial pressure of oxygen, indicating a reduction in cardiac output and oxygen delivery to tissues (Nyman *et al.*, 2000).

1.3. Alpha-2-adrenergic receptor antagonists

Alpha-2-antagonists function by competitively binding to the alpha-2-adrenergic receptor, thereby hindering its activation. This action leads to the reversal of the physiological effects induced by alpha-2-agonists, which varies depending on the species and specific drug (Posner, 2018). Notably, alpha-2-antagonists are employed to counteract the sedation caused by alpha-2-agonists. Commonly used antagonists include Yohimbine, tolazoline, and atipamezole. In veterinary medicine, these drugs are primarily indicated for reversing sedation and off-label treatment of intoxication from certain substances (McMurphy *et al.*, 2018).

However, the abrupt and complete reversal of an agonist can trigger a sudden surge in sympathetic activity and heightened pain sensation (Pertovaara *et al.*, 2005). Pertovaara *et al.* (2005) observed that atipamezole, in particular, obstructed the NE feedback inhibition of pain perception, thereby intensifying pain sensation. Additionally, alpha-2-antagonists exhibit varying selectivities in binding to alpha-1 and alpha-2 receptors (Posner, 2018). Tolazoline, for instance, demonstrates an equal affinity for both receptor types. In contrast, Yohimbine exhibits greater selectivity for alpha-2 receptors over alpha-1 receptors, while atipamezole surpasses Yohimbine in alpha-2 selectivity (Dugdale *et al.*, 2020). Yohimbine's use in veterinary medicine has waned since the introduction of atipamezole to the market (McMurphy *et al.*, 2018).

Tolazoline, a synthetic antagonist, is utilized in horses to reverse xylazine-induced sedation. However, its efficacy against detomidine sedation is partial and short-lived (McMurphy *et al.*, 2018). On the other hand, atipamezole, an imidazole derivative, is effective in reversing medetomidine and dexmedetomidine, as well as other alpha-2-agonists. Apart from reversing sedation, atipamezole administration may provoke muscle tremors, tachycardia, transient hypotension, panting, defecation, vomiting, and heightened alertness. Furthermore, atipamezole's interference with spinal opioid effectiveness has been noted (Dugdale *et al.*, 2020). Despite its use, atipamezole may not fully counteract the cardiovascular effects induced by alpha-2-agonists (McMurphy *et al.*, 2018; Dugdale *et al.*, 2020).

The timing of atipamezole administration relative to alpha-2-agonist injection significantly impacts its effectiveness. Administering the antagonist too early may result in the ineffectiveness of the agonist, as atipamezole competes with the agonist for receptor occupancy. Moreover, atipamezole, not licensed for equine use, should be administered either

intramuscularly or via slow IV injection to mitigate the risk of sudden hypotension and tachycardia associated with rapid injection (Dugdale *et al.*, 2020). Additionally, Dugdale *et al.* (2020) suggest that atipamezole may diminish sedation in horses.

1.3.1. Vatinoxan

Vatinoxan also referred to as MK-467 and L-659,066, functions as a selective peripherally acting alpha-2-antagonist. Studies indicate its limited ability to penetrate the blood-brain barrier, rendering it ineffective in the CNS. This was demonstrated through oral and IV administration of radioactively labelled derivatives of vatinoxan, with subsequent calculation of brain/plasma ratios at various intervals, revealing low accumulation in the brain (Clineschmidt *et al.*, 1988).

In a study involving sheep conducted by Adam *et al.* (2021), plasma concentrations of vatinoxan surpassed those in the brain and cerebrospinal fluid, underscoring its poor blood-brain barrier permeability. Similar findings were observed in dogs by Honkavaara *et al.* (2020), where CNS concentrations of vatinoxan were merely 2 % of plasma levels. Additionally, vatinoxan failed to inhibit clonidine-induced mydriasis or elicit a rise in dopamine levels in rats (Clineschmidt *et al.*, 1988).

Assessment of vatinoxan's postsynaptic effects involved its ability to block UK 14,304-induced increases in diastolic pressure, while its presynaptic effects were evaluated through inhibition of clonidine-induced heart rate elevation, both of which it successfully antagonized. The observed poor blood-brain barrier penetration was attributed to vatinoxan's low lipophilicity (Clineschmidt *et al.*, 1988).

Adam *et al.* (2021) suggested cerebrospinal fluid as a potential alternative for determining vatinoxan concentration in the brain, given the comparable concentrations observed in their study. Furthermore, Clineschmidt *et al.* (1988) noted vatinoxan's high affinity solely to alpha-2-adrenergic receptors, with minimal interaction with other receptor types (e.g. dopaminergic, serotonergic, beta-adrenergic, and muscarinic). Its selectivity to alpha-2-receptors compared to alpha-1-receptors was tested by assessing vatinoxan's ability to antagonize methoxamine (an alpha-1-agonist) and clonidine (an alpha-2-agonist) effects on vas deferens, having a weaker effect on methoxamine (Clineschmidt *et al.*, 1988).

Vatinoxan's impact on the GI tract was assessed via the colonic propulsion test in rats, demonstrating its ability to inhibit clonidine-induced expulsion of glass beads (Clineschmidt *et al.*, 1988). In a study on dogs by Honkavaara *et al.* (2012), different treatment combinations involving dexmedetomidine (0,01mg/kg) and vatinoxan (0,25 mg/kg alone; or 0,25; 0,5; or 0,75 mg/kg with dexmedetomidine) were administered, yielding specific pharmacokinetic parameters for vatinoxan alone. The volume of distribution of vatinoxan (0,25 mg/kg) was 0.41 ± 0.13 L/kg, clearance was $7,8 \pm 3,4$ mL/kg/min, and elimination half-life was $39 \pm 7,6$ minutes (Honkavaara *et al.*, 2012). The elimination half-life of vatinoxan in horses with the dosage of 0,2 mg/kg is 141 ± 28.6 minutes, the volume of distribution is 1.189 ± 0.121 L/kg and clearance is $6,0 \pm 0,99$ mL/kg/min (de Vries *et al.*, 2016).

In horses, administration of vatinoxan alone led to behavioural stimulation, characterized by restlessness, and increased heart and respiratory rates, as reported by de Vries *et al.* (2016). It was suggested that this effect was attributed to the removal of presynaptic inhibition of autoreceptors. Pakkanen *et al.* (2018) observed mild abdominal discomfort and occasional watery stool in horses following vatinoxan administration.

1.3.2. Combining vatinoxan with alpha-2-agonists

The co-administration of vatinoxan with alpha-2-agonists has been investigated in the context of sedation in dogs and horses, as documented by several studies (Honkavaara *et al.*, 2008; Pakkanen *et al.*, 2018; Tapio *et al.*, 2018; Tapio *et al.*, 2019; Hallman *et al.*, 2024). While the majority of these studies indicate that the combination does not significantly alter sedation levels, a subset of research, including Restitutti *et al.* (2011), Vainionpää *et al.* (2013), and de Vries *et al.* (2016), suggests a marginal decrease in sedation with the inclusion of vatinoxan in the premedication protocol for dogs and horses.

Bennett *et al.* (2016) observed a shorter duration of sedation induced by medetomidine when vatinoxan was administered concurrently in dogs. Additionally, de Vries *et al.* (2016) proposed an increase in romifidine dosage when combined with vatinoxan to achieve a clinically sufficient level of sedation. In a study by Honkavaara *et al.* (2008), where dogs were sedated with dexmedetomidine alone or in combination with vatinoxan, the subsequent reversal of sedation using atipamezole showed rapid and calm recovery, unaffected by vatinoxan.

Bennett *et al.* (2016) reported shorter limb withdrawal and head lift times in dogs with vatinoxan (0.25 mg/kg, IV) compared to medetomidine (0.01 mg/kg, IV) alone, indicating an impact on the antinociception induced by medetomidine. However, when comparing these times to the baseline, the addition of vatinoxan to the sedation protocol did not yield significant differences, possibly attributed to a suboptimal medetomidine dose for sufficient analgesia.

On the contrary, Ulger *et al.* (2009) found that vatinoxan (1 mg/kg, IV) did not affect dexmedetomidine (0.0025-0.02 mg/kg, IV) induced antinociception in rats. Similar results were obtained by Huuskonen *et al.* (2020) in dogs receiving IV medetomidine (0.02 mg/kg) alone or in combination with vatinoxan (0.02 mg/kg or 0.04 mg/kg of medetomidine and 0.4 mg/kg or 0.8 mg/kg of vatinoxan).

Certain studies, such as those by Pakkanen *et al.* (2014) and Tapio *et al.* (2019), have reported that vatinoxan contributes to a reduction in anaesthesia induction time when used as premedication in conjunction with an alpha-2-agonist.

1.3.2.1. Pharmacokinetics

Several studies provide conflicting evidence regarding the impact of vatinoxan on the pharmacokinetics of alpha-2-agonists. Tapio *et al.* (2018) suggest that vatinoxan has minimal effect on the pharmacokinetics of alpha-2-agonists, particularly when administered 10 minutes after detomidine. Conversely, Vainionpää *et al.* (2013) found that concurrent administration of detomidine and vatinoxan led to a reduction in detomidine's sedative effect, attributed to faster distribution and clearance facilitated by vatinoxan.

In contrast to Vainionpää *et al.* (2013), Tapio *et al.* (2018) observed no significant changes in detomidine plasma concentrations when combined with vatinoxan, although they administered vatinoxan post-detomidine administration, thus potentially mitigating its impact during initial minutes. Pakkanen *et al.* (2014) demonstrated that vatinoxan, when added to premedication, decreased detomidine and butorphanol plasma concentrations, indicating enhanced systemic clearance. Similarly, de Vries *et al.* (2016) proposed that vatinoxan accelerated drug distribution and clearance, affecting the pharmacokinetics of romifidine by altering its half-life, volume of distribution, and clearance rate.

Furthermore, in a sheep study by Adam *et al.* (2021), vatinoxan did not influence xylazine distribution, albeit sedation duration was not monitored beyond euthanasia. Honkavaara *et al.*

(2012) noted a doubling of dexmedetomidine's volume of distribution in dogs with vatinoxan administration.

1.3.2.2. Cardiovascular effects

The efficacy of vatinoxan in mitigating alpha-2-agonist-induced bradycardia in horses has been well-established through multiple studies (Vainionpää *et al.*, 2013; Pakkanen *et al.*, 2014; de Vries *et al.*, 2016; Tapio *et al.*, 2018). Furthermore, Vainionpää *et al.* (2013) demonstrated the preventive effects of vatinoxan on second-degree atrioventricular blocks induced by alpha-2-agonists. Notably, the hypertensive response initiated by detomidine was attenuated by vatinoxan (Vainionpää *et al.*, 2013). Additionally, Tapio *et al.* (2018) revealed the reversal of alpha-2-agonist-induced baroreflex by vatinoxan, resulting in the alleviation of vasoconstriction.

Combining alpha-2-agonists with vatinoxan led to an elevated heart rate in horses (Pakkanen *et al.*, 2014; de Vries *et al.*, 2016). Administration of vatinoxan alone exhibited an increase in heart rate and restlessness, possibly attributed to heightened sympathetic nervous system activity, as postulated by de Vries *et al.* (2016). The precise mechanism for these effects remains unclear (de Vries *et al.*, 2016).

Studies incorporating vatinoxan with alpha-2-agonists as premedication in isoflurane anaesthesia revealed instances of hypotension in horses, necessitating increased dobutamine usage to maintain normal blood pressure (Pakkanen *et al.*, 2014; Tapio *et al.*, 2019). The mechanisms proposed by Pakkanen *et al.* (2014) include potential increased dobutamine clearance or vatinoxan counteracting medetomidine-induced vasoconstriction, leading to reduced mean arterial pressure and heightened systemic vascular resistance, as suggested by Tapio *et al.* (2019). Pakkanen *et al.* (2014) also hypothesized that vatinoxan-induced hypotension may result from either isoflurane-induced reduction in arterial blood pressure or vatinoxan-induced decrease in mean arterial pressure overpowering detomidine-induced vasoconstriction. Furthermore, Tapio *et al.* (2019) reported that vatinoxan influenced haemoglobin, arterial oxygen content, and mixed venous oxygen content by augmenting their levels. The observed increases in haemoglobin and arterial oxygen content were attributed to dobutamine and splenic contractions, while the rise in mixed venous blood oxygen content was linked to elevated haemoglobin concentrations (Tapio *et al.*, 2019).

De Vries *et al.* (2016) suggested that the dose of vatinoxan could be optimized to prevent alpha-2-agonist-induced cardiac disturbances, although their study involved bolus administration rather than CRI under isoflurane anaesthesia.

1.3.2.3. Respiratory system

In an investigation conducted by Pakkanen *et al.* (2014), equines were subjected to premedication with butorphanol in conjunction with either detomidine alone or detomidine combined with vatinoxan. Anaesthesia induction was achieved using ketamine and midazolam, followed by maintenance with isoflurane. Dobutamine infusion was administered to provide hemodynamic support. The findings indicated that the concurrent administration of vatinoxan with detomidine resulted in elevated arterial and venous partial pressure of oxygen, as well as an increased ratio of arterial oxygen tension to inspired oxygen compared to the administration of detomidine alone. The observed improvement was attributed to enhanced cardiac function and mitigation of ventilation-perfusion mismatch facilitated by vatinoxan (Pakkanen *et al.*, 2014).

Similarly, De Vries *et al.* (2016) reported a reduction in respiratory rates in horses sedated with either romifidine alone or in combination with vatinoxan. This decrease was posited to arise from either the sedative effects of romifidine, a reduction in ventilatory responsiveness, or a diminished sensitivity of receptors (De Vries *et al.*, 2016).

1.3.2.4. Gastrointestinal effects

Vatinoxan has demonstrated efficacy in mitigating the alpha-2-agonist-induced reduction in GI motility, as assessed through the auscultation of borborygmi in equines (Vainionpää *et al.*, 2013; de Vries *et al.*, 2016; Tapio *et al.*, 2018). Vainionpää *et al.* (2013) observed that vatinoxan effectively maintained GI motility, counteracting the suppressive effects typically induced by detomidine. Tapio *et al.* (2018) reported an expedited onset of defecation in horses treated with vatinoxan compared to those administered saline. However, it should be noted that there was no significant variance in total faecal output when vatinoxan was combined with an alpha-2-agonist (Tapio *et al.*, 2018; 2019).

Pakkanen *et al.* (2018) documented mild abdominal discomfort and the production of watery faeces following the administration of vatinoxan in isolation. Similar findings were reported by de Vries *et al.* (2016), who proposed that these effects were attributable to an augmentation

in intestinal motility. Additionally, de Vries *et al.* (2016) observed that the administration of vatinoxan alone did not impact borborygmi.

1.3.2.5. Glucose and insulin

Pakkanen *et al.* (2018) observed that vatinoxan mitigated the alpha-2-agonist-induced elevation in plasma glucose concentration in their investigation, albeit without complete prevention. The investigators posited that the potential prevention of hyperglycaemia might be achievable through a dosage escalation of vatinoxan, given its dose-dependent nature. Alternatively, they proposed that peripheral alpha-2-adrenergic receptors may only partially regulate glucose homeostasis. Independent administration of vatinoxan did not induce alterations in glucose levels but led to a reduction in serum insulin concentrations. The observed impact on insulin was attributed to the inhibition of insulin secretion from the pancreas and the activation of the sympathetic nervous system in response to stress (Pakkanen *et al.*, 2018). In contrast, Tapio *et al.* (2018) reported that the concurrent administration of vatinoxan and an alpha-2-agonist did not result in significant deviations in plasma glucose levels from baseline.

2. AIM OF THE STUDY

The aim is to evaluate the effect of vatinoxan on GI motility in horses sedated with a four-hour detomidine CRI. The hypothesis is that vatinoxan inhibits the negative side effects of detomidine in the GI tract.

3. MATERIALS AND METHODS

3.1. Animals, housing, and feeding

Eight clinically healthy adult Finnhorses, owned by the Natural Resources Institute Finland and Harju Vocational College, were carefully selected for this study. Prior to inclusion, each horse underwent a thorough clinical examination and routine laboratory tests to ensure its health status. The group comprised six mares and two geldings, aged between four and 15 years, with a median age of 13 years. The horses exhibited a mean weight of 591 kg, ranging from 550 kg to 620 kg.

Before the study commenced, the horses resided in an open loose housing system with separate sections for mares and geldings. However, during the study period, they were individually housed in stalls with peat bedding within a stable. Daily, from 12 pm to 4 pm, on the second and third days after the end of CRI, the horses were given access to the paddock for four hours, during which no samples were collected, and the horses were not monitored.

Until the morning of the study, the horses were fed with an automatic feeding system, after which the researchers took over the feeding regimen. Throughout the research, the horses had free access to water and straw. Their daily diet comprised approximately 10 kg of hay, divided into five feedings of approximately 2 kg each. Additionally, they received 0.5 L of mash diluted in 10 L of water once a day, oat grains ranging from 50 to 1000 g per day (median: 100 g), and approximately 4 dL of minerals. Feed was initially offered about two hours after the conclusion of the CRI or until the horse fully regained consciousness. In case of colic symptoms, feeding was restricted, and the horses were walked until the symptoms subsided. Only one horse exhibited signs of colic after the end of CRI.

3.2. Study design

Each horse received two treatments in a randomized order, with a minimum interval of three weeks between treatments.

3.3. Instruments and sedation protocol

On the study morning, the horses were weighed and placed in stocks for clinical examination and sample collection. Various physiological parameters were assessed, including heart rate (via auscultation), respiratory rate (observation of lateral body wall movement), body temperature, skin turgor, borborygmus score (described below), digital pulse, mucous membrane colour, capillary refill time, and jugular filling on both sides of the neck.

IV catheters (12 G Intraflon, Génia, France) were aseptically placed in both jugular veins for drug administration and blood sampling. After each bolus administration and blood sample collection, the catheters were flushed with heparin (5 mL of heparin per 1000 mL of sodium chloride, Heparin Leo 5000 IU/mL, Leo Pharma, Denmark) to prevent clotting and subsequently removed.

Vatinoxan hydrochloride, initially in powdered form, was diluted to a 10 mg/mL solution with saline. An initial IV bolus of 0.01 mg/kg of detomidine hydrochloride (Equisedan 10 mg/mL, Vetcare Ltd., Finland) alone or in combination with 0.15 mg/kg vatinoxan hydrochloride (Vetcare Ltd., Finland) was administered, followed by a CRI of detomidine (0.015 mg/kg/h) with or without vatinoxan (0.05 mg/kg/h) using infusion pumps (B. Braun, Melsungen, Germany) for four hours.

A nasogastric tube was inserted after the bolus, and 200 small-coloured plastic spheres (diameter 5.95 mm; weight 0.2 g) with 4 L of water were administered. A nose twitch was utilized to calm the horses during catheterization and nasogastric tube insertion.

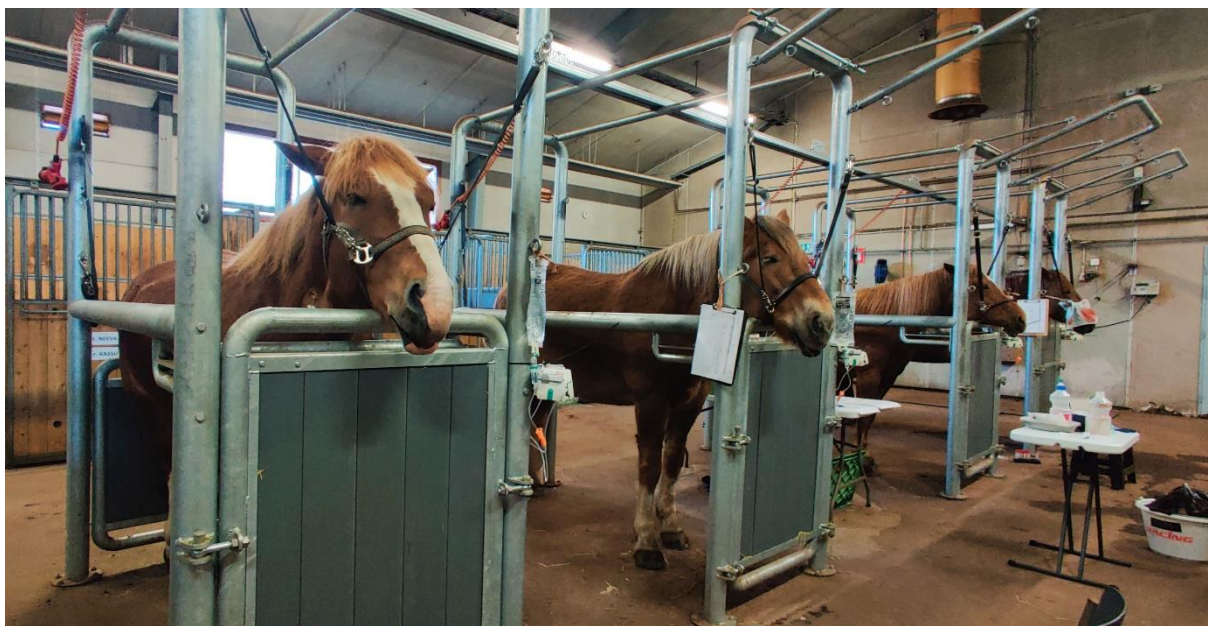


Figure 1. Horses were placed in the stocks under a four-hour CRI with either detomidine alone or detomidine in combination with vatinoxan.

3.4. Monitoring of GI motility

Intestinal sounds were auscultated using a stethoscope by a blinded observer and scored according to a scoring system (Mama *et al.*, 2009) before the initial bolus (basal data) and

thereafter at time points 30, 60, 120, 180, 240 minutes after the start of CRI and 60 minutes after the end of CRI. Each quadrant (consisting of the upper and lower flank on both the left and right sides) was auscultated for 30 seconds, and a numerical score equal to the number of borborygmi within that duration was given. If uncoordinated rumbling or gaseous sounds were auscultated, a score of 0,5 was assigned. Scores from all quadrants were summed up to provide a single score for GI motility. Time 0 was considered the time when base data was recorded (before the initial bolus).

The time of the first defecation after the start of CRI was recorded. All faeces produced during a seventy-two-hour period after the end of the CRI were collected, weighed, and counted every four hours (excluding four hours per day when the horses were in the loose housing system on the second and third days). The frequency of defecation was determined by the number of piles produced in a four-hour interval. The weight of the faeces represented the cumulative weight of all piles during that duration. To determine the intestinal transit time the passage of plastic spheres into faeces was assessed. Starting from 12 hours after the end of CRI and continuing until 72 hours post-CRI, the plastic spheres were collected and counted from the faeces every four hours. The collection and counting process was manually performed by the researchers. Time 0 for intestinal transit time, frequency of defecation, and faecal weight was the end of CRI.

In addition, heart rate was auscultated during the sedation, but the results are not reported in this study.

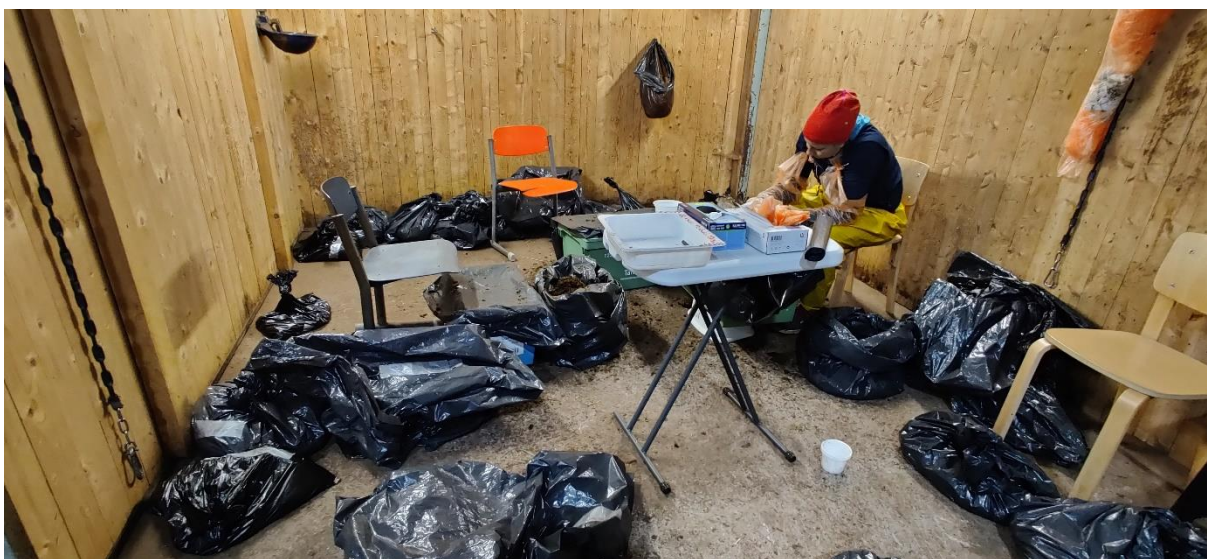


Figure 2. Manual collection and counting of the plastic spheres from the faeces.

3.5. Statistical analysis

Data management and analysis for borborygmus scores and the times for the first defecations were performed using IBM SPSS Statistics version 29.0. Data management and analysis for faecal output, frequency of defecation, and intestinal transit time Microsoft Excel for Microsoft 365 Version 2403. Borborygmus scores and time for the first defecation were analysed using the Related-Samples Wilcoxon Signed Rank test with a 95 % confidence interval, and the p-value being equal to or under 0.05. Mean and range were calculated for the time of the first defecation. Faecal output, frequency of defecation, and intestinal transit time were analysed with Student's t-test with a 95 % confidence interval, and the p-value was equal to or under 0.05.

3.6. Ethics

The National Animal Experimental Board of Finland accepted this study on August 11th, 2022, ESAVI/23464/2022. The author confirms the absence of a conflict of interest. The findings were not affected by the pharmaceutical company Vetcare Ltd. The company supported the present study by funding it and donating vatinoxan hydrochloride and detomidine hydrochloride that were administrated to the animals participating in the study.

4. RESULTS

The comparison of the borborygmi over a time window of 480 minutes showed that following the start of the CRI, there was a significant decline in the average borborygmus scores with detomidine alone compared to its base value. The borborygmus score remained consistent until 240 minutes (the end of the infusion) and then began to increase slowly. Despite this slow recovery, the levels never reached the base value (Figure 3; $p \leq 0.05$).

In the presence of vatinoxan, the time-dependency of borborygmi showed a less steep reduction of borborygmi 30 minutes after the start of the CRI compared to detomidine alone. With vatinoxan, the borborygmus score was significantly reduced from 60 minutes after the start of the infusion until the end of the infusion compared to its base value (Figure 3; $p \leq 0.05$). The values subsequently gradually returned to the base value.

There was a statistically significant difference between the treatment with detomidine alone and detomidine in combination with vatinoxan (Figure 3; $p \leq 0.05$).

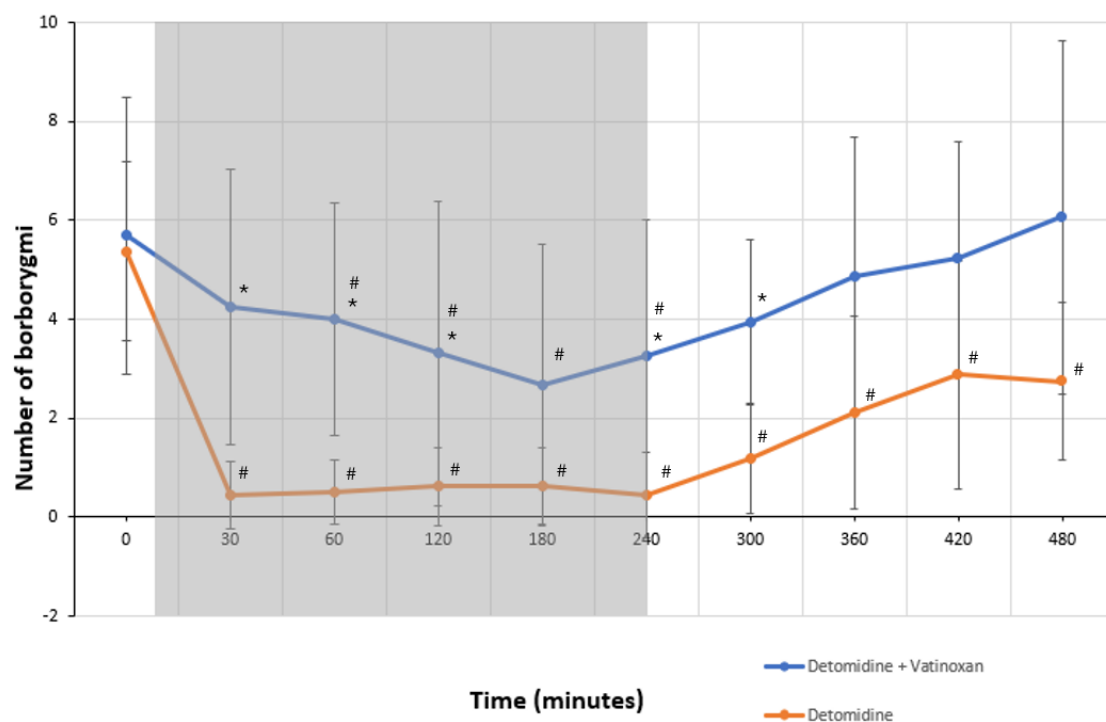


Figure 3. Average borborygmus in horses receiving treatment with detomidine alone or detomidine in combination with vatinoxan. Asterisks indicate the statistically significant difference between the two treatments with a 95 % confidence interval ($p_{30 \text{ and } 60 \text{ min}}=0.02$; $p_{120 \text{ min}}=0.04$; $p_{240 \text{ min}}=0.04$; $p_{300 \text{ min}}=0.03$), $n=8$. Hashtags indicate the statistically significant

difference between the treatment and its base values with a 95 % confidence interval (detomidine in combination with vatinoxan: $p_{60, 120, \text{ and } 180\text{min}}=0.02$; $p_{240\text{min}}=0.01$; detomidine alone: $p_{30-300\text{min}}=0.01$; $p_{360\text{min}}=0.02$; $p_{420\text{min}}=0.03$; $p_{480\text{min}}=0.02$), $n=8$. The gray area indicates the time of infusion.

The average total faecal output was not significantly different between the two treatments ($p=0.5$). When assessing the faecal output per time point it was observed that the output with vatinoxan-treated horses defecated statistically different than with detomidine alone only at the beginning of the measurement period after the end of CRI (Figure 4; $p_{4\text{h}}=0.002$; $p_{8\text{h}}=0.03$).

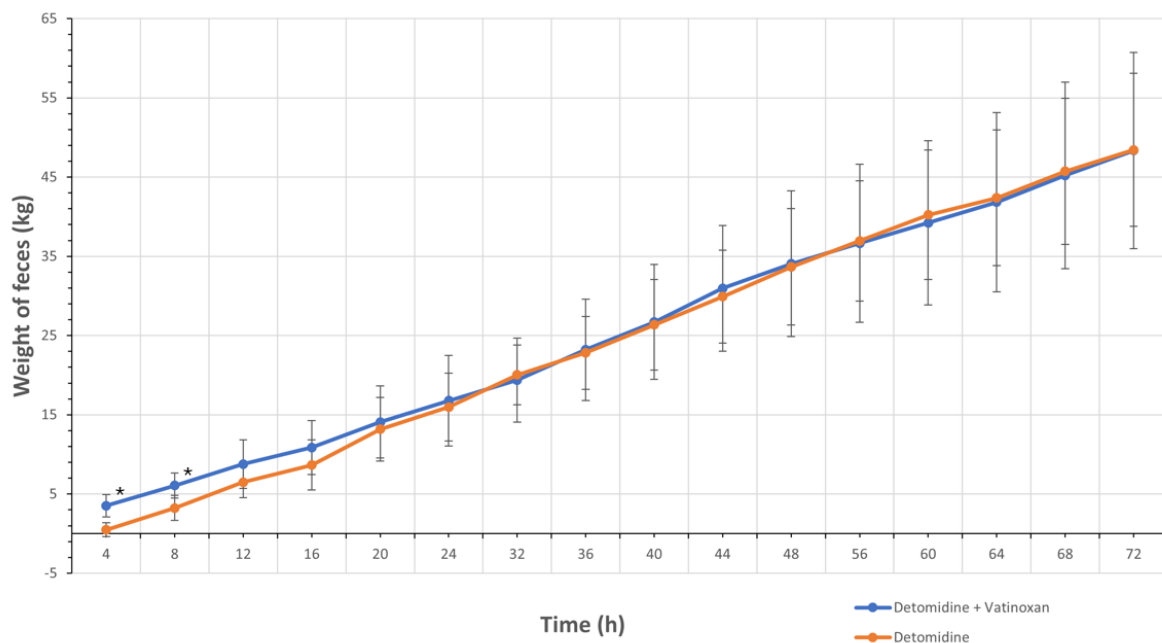


Figure 4. The cumulative weight of faeces with the treatment of detomidine alone and detomidine in combination with vatinoxan. The asterisks indicate the statistically significant difference with a 95 % confidence interval ($p \leq 0.05$), $n=8$.

A comparison of the total cumulative number of faecal piles over the 72-hour measurement period did not show a significant difference between the two treatments (Figure 5; $p=0.8$). When assessing the cumulative number of faecal piles at individual time points it was observed that with vatinoxan-treated horses the frequency of defecation was statistically higher than with detomidine alone only 4 and 8 hours after the end of CRI ($p_{4\text{h}}=0.004$; $p_{8\text{h}}=0.02$).

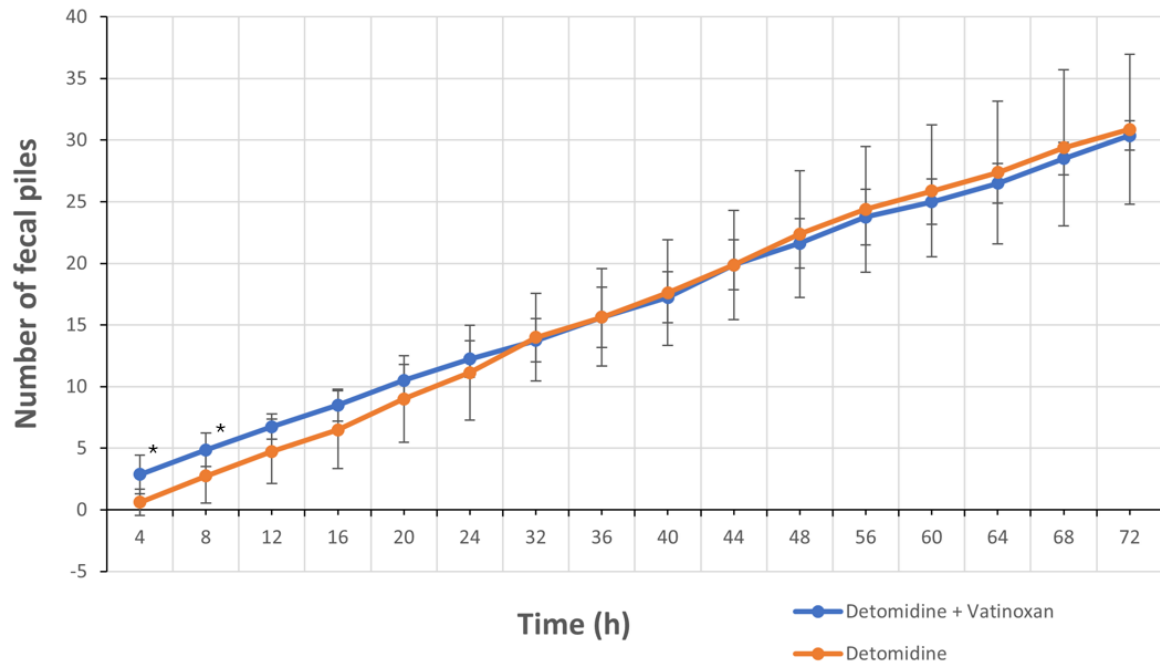


Figure 5. The cumulative frequency of defecation with the treatment of detomidine alone or detomidine in combination with vatinoxan. The asterisks indicate the statistically significant difference between the treatments with a 95 % confidence interval ($p \leq 0.05$), $n=8$.

The time in which the highest count of spheres was expelled with the faeces was around the same time points for both treatments. For detomidine alone, it was 32 hours after the end of CRI, and for detomidine in combination with vatinoxan, it was 36 hours after the end of CRI (Tabel 1). When comparing the cumulative values between the two treatments there was a significant difference between 44 to 60 hours after the end of CRI (Figure 6).

Table 1. The average count of coloured spheres and their standard deviations in horses treated with detomidine alone or in combination with vatinoxan. 200 spheres were administered after the initial bolus

Time (h)	Detomidine	Detomidine + vatinoxan
12	0.1 ± 0.3	0.1 ± 0.4
16	0.3 ± 0.5	0.3 ± 0.7
20	3.6 ± 4.6	0.9 ± 2.3
24	5.1 ± 4.9	2.5 ± 4.3
32	30.4 ± 23.3	8.0 ± 4.0
36	17.5 ± 17,3	17.6 ± 19.8
40	18.1 ± 10.6	9.8 ± 4.1
44	18.5 ± 10.9	11.9 ± 10.4
48	13.3 ± 12.2	9.8 ± 7.0
56	7.3 ± 5.7	9.5 ± 5.5
60	3.8 ± 3.2	6.1 ± 7.6
64	1.8 ± 1.8	6.5 ± 8.9
68	2.4 ± 2.3	8.4 ± 6.0
72	2.1 ± 2.9	7.1 ± 7.0

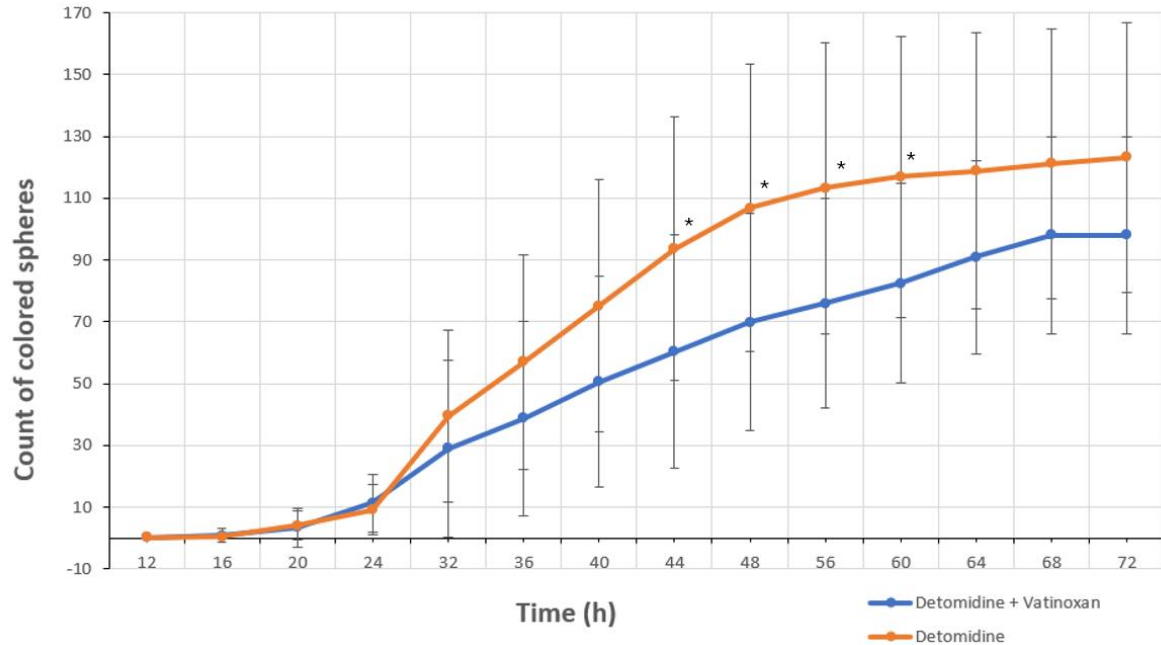


Figure 6. The cumulative count of plastic spheres in the faeces of horses treated with detomidine alone or with detomidine in combination with vatinoxan. The asterisks indicate the statistically significant difference between the treatment groups with a 95 % confidence interval ($p_{44h}=0.02$; $p_{48h}=0.04$; $p_{56h}=0.03$; $p_{60h}=0.04$), $n=8$.

There was a statistically significant difference in times of the first defecation in horses treated with detomidine alone or in combination with vatinoxan ($p=0.01$; $n=8$). The mean time for the first defecation with detomidine alone was 10.5 h (8-12 h) and with detomidine in combination with vatinoxan was 4.6 h (0.5-8 h).

5. DISCUSSION

In alignment with previous research (Mama *et al.*, 2009; Vainionpää *et al.*, 2013; Tapio *et al.*, 2018), this study confirms that detomidine alone significantly reduced borborygmus scores compared to the base value. The effect persisted throughout the measurement period and remained evident even four hours after the end of CRI, without returning to the base value. However, when detomidine was administered alongside vatinoxan the reduction in borborygmi scores compared to baseline was less pronounced between 30- and 240-minutes post-infusion commencement. Unlike in this study, Tapio *et al.* (2018) observed a significant decrease only within the first 5 to 30 minutes following an IV injection of vatinoxan, which was administered 10 minutes after a detomidine bolus. Instead, Vainionpää *et al.* (2013) reported no significant difference of borborygmi scores from baseline in horses receiving an IV bolus of detomidine in combination with vatinoxan. The comparison of the treatment protocols in this study revealed a significant difference in borborygmi scores, suggesting that vatinoxan counters the detomidine-induced reduction in borborygmi. This aligns with findings by Vainionpää *et al.* (2013) with detomidine and vatinoxan, as well as by de Vries *et al.* (2016) with romifidine and vatinoxan. Interestingly, one horse in this study exhibited a contrasting response to detomidine when combined with vatinoxan, experiencing a more pronounced reduction in borborygmi compared to others.

Faecal output and frequency of defecation were significantly higher when vatinoxan was coadministered during the first 8 hours after the end of CRI. However, the total faecal output over 72 hours did not differ between treatments. Similarly, Tapio *et al.* (2018) reported no difference in total faecal output over 24 hours between treatments. However, our study did not evaluate baseline values for faecal output or defecation frequency without sedation, preventing direct comparison of vatinoxan and detomidine effects on these parameters against control conditions. Notably, in this study the faecal samples were collected more frequently than in Tapio *et al.*'s (2018) study, potentially enhancing accuracy.

Consistent with Vainionpää *et al.* (2013) and Tapio *et al.* (2018), horses in this study passed faeces sooner when vatinoxan was combined with detomidine compared to detomidine alone. However, the time to first defecation was nearly tripled with detomidine alone in our study compared to Tapio *et al.* (2018) and doubled with vatinoxan. This difference may be due to the

differing administration methods; this study employed a 4-hour infusion, whereas Tapio *et al.* (2018) utilized bolus injections whose effect is expected to be of a shorter duration.

To the author's knowledge, the effects of vatinoxan on intestinal transit time in horses have not been previously studied. Contrary to the prior results, fewer coloured spheres were expelled with vatinoxan compared to detomidine alone, suggesting that the combination slowed GI motility more than detomidine alone, which is somewhat conflicting. Not all spheres were recovered during the measurement period with either treatment. With detomidine alone 61 %, and with vatinoxan only 49 % of all spheres were recovered indicating that a longer measurement period might be needed. Previous studies utilizing similar methods reported higher recovery rates (Lippold *et al.*, 2004; Boscan *et al.*, 2006), suggesting room for improvement in this methodology.

Several factors may have contributed to the low recovery of the spheres observed in this study. It is plausible that some spheres were lost when the horses were released into the paddock twice during the measurement period, totalling eight hours. Additionally, researchers may have accidentally missed some spheres during manual inspection of the faecal samples, and some could have been lost in the peat bedding within the stalls. Furthermore, the level of physical activity in the paddock versus the stalls may have also influenced intestinal transit time, as noted by Orton *et al.* (1985).

The size and characteristics of the spheres are known to impact their transit time through the GI tract, as reported by Hinton *et al.* (1969) and Orton *et al.* (1985). Additionally, it remains unclear whether the spheres moved alongside liquid or solid matter, which could have affected their transit time differently. The composition of the horse's diet, such as water and straw, may have also influenced GI transit time (Argenzio *et al.*, 1974).

CONCLUSIONS

The administration of alpha-2-agonists in horses is known to reduce GI motility, potentially increasing the risk of post-sedation complications such as ileus, impaction, or colic (Zullian *et al.*, 2011; Vainionpää *et al.*, 2013; Tapio *et al.*, 2018). To address this issue, the concurrent administration of a peripherally acting alpha-2-antagonist, vatinoxan, presents a promising solution to mitigate these side effects without compromising the sedation itself. In our study, the combination of vatinoxan and detomidine resulted in a milder reduction in borborygmi score and quicker passage of faeces in horses. This suggests the potential of vatinoxan to mitigate the adverse effects associated with prolonged detomidine infusion. However, the lower excretion of plastic spheres with vatinoxan treatment presents a conflicting aspect thus further studies are required to investigate this difference.

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APPENDICES

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