THE EFFECT OF SEDATION ON THE SUCROSE PERMEABILITY CURVE

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Tämä lisensiaatin tutkimus koostuu kolmesta osasta: kirjallisuuskatsauksesta, kokeellisesta osasta ja liitteistä.

Hevosten mahahaavat ovat yleinen sairaus. Maha-suolikanavan mukoosa on vahingoittunut ja haavauma voi yltää lamina propriaan asti. Sairauden diagnosointi tapahtuu tyypillisesti kliinisten oireiden ja hoitovasteen perusteella. Ainoa varma diagnosointimenetelmä on mahan tähystys. Tähystys on kallista ja vaatii erityistaitoa, jonka vuoksi olisi tarve löytää vaihtoehtoinen diagnosointimenetelmä.

Terve suoliston mukoosa ei läpäise sakkaroosia ja se hydrolysoidaan ohutsuolessa monosakkarideiksi, joka imeytyy ohutsuolen mukoosan läpi. Jos hevosella on mahahaava, mukoosa on vahingoittunut ja sakkaroosi pääsee imeytymään sen läpi. Imeytynyt sakkaroosi voidaan todeta veressä. Tämän perusteella seerumin sakkaroosi pitoisuuden mittaaminen voisi olla hyvä vaihtoehto mahahaavan diagnosointiin. Sakkaroosin pitoisuus mahdollisesti myös korreloi mahahaavan vakavuuden kanssa. Tämän hypoteesin selvittämiseksi on suunnitteilla tutkimus, jossa suuri määrä hevosia tähystetään, jonka jälkeen niille annostellaan sakkaroosia ja otetaan verinäyte. Verikokeista määritellään sakkaroosin määrä ja verrataan tulosta mahahaavan vakavuuteen.

Tähystystä varten hevoset on rauhoitettava. Yleisimmin hevosen rauhoituksessa käytetään detomidiniä ja butorphanolia. Useiden tutkimusten mukaan nämä aineet vaikuttavat mahalaukun tyhjentymiseen hidastavasti. Jos mahalaukun tyhjentyminen hidastuu, sakkaroosi pysyy mahdollisesti pidempään mahalaukussa, mikä voi vaikuttaa sakkaroosin pitoisuuteen seerumissa. Sakkaroosin pitoisuuteen seerumissa voisi siis vaikuttaa se, onko hevonen rauhoitettu vai ei. Tämän tutkimuksen tavoite onkin selvittää rauhoituksen vaikutusta sakkaroosin imeytymiseen .

Materiaalimme koostui kymmenestä terveestä hevosesta. Kaikille hevosille suoritettiin kliininen tutkimus ja niiltä otettiin verinäytteet maha-suolikanavan sairauden poissulkemiseksi. Ensimmäisessä kokeessa hevosille annosteltiin 16 tunnin paaston jälkeen 500 grammaa sakkaroosia 10 % -liuoksena nenämahaletkulla suoraan mahaan ja verinäytteet otettiin 0,45, 90, 135, 180 ja 225 minuuttia annostelun jälkeen. Seuraavassa kokeessa hevoset rauhoitettiin 16 tunnin paaston jälkeen ja niille suoritettiin mahalaukun tähystys. Tämän jälkeen niille annosteltiin sakkaroosi ja otettiin verikokeet ensimmäisen kokeen mukaisesti. Sakkaroosin pitoisuus veressä määritettiin GC-FID metodilla. Sakkaroosin pitoisuudesta tietyillä ajanhetkillä hevosen ollessa rauhoitettuna ja ei-rauhoitettuna muodostettiin kuvaajat.

Sakkaroosi voitiin havaita seerumissa 45 minuutin kuluttua annostelusta ja sitä oli vielä havaittavissa 225 minuutin kohdalla. Ei-rauhoitetuilla hevosilla sakkaroosin seerumipitoisuudet olivat suuremmat kaikkina mittaus ajankohtina. Kuvaajan malli oli sama kummallakin ryhmällä. Ei-rauhoitetuilla hevosilla huippu tuli jo 45 minuutissa, kun se rauhoitetuilla hevosilla tuli 90 minuutissa.

Rauhoitetuilla hevosilla sakkaroosin imeytyminen oli hitaampaa. Tähän selityksenä voi olla tähystys ja siihen liittyvä mahalaukun täyttäminen ilmalla, joka suoritettiin ainoastaan rauhoitetuilla hevosilla. Kuvaajien muotojen yhteneväisyyden perusteella sakkaroosin seerumipitoisuutta voidaan verrata rauhoitetuilla ja ei-rauhoitetuilla hevosilla, mutta viiterajojen pitää olla kummallakin ryhmällä erilaiset.

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PREFACE

This presentation includes three sections: a literature review, a research project and appendixes. Appendix I is a health assessment sheet which was used in the clinical examination. Appendix II is the results of the clinical examinations. In appendix III there are the results of serum biochemistries and complete blood counts.

1 INTRODUCTION

Gastric ulceration is a common disease in horses. The gastrointestinal mucosa is damaged and the ulcer can extend to the level of the lamina propria. (The Equine gastric ulcer council 1999.) Gastric ulcers cause a variety of clinical signs including weight loss, poor body condition, lack of appetite, loose faeces and mild or recurrent colic. The disease is typically diagnosed by clinical signs and response to therapy. Gastroscopy is at the moment the only method for definitive diagnosis. (Bell et al. 2007.) Gastroscopy is expensive and needs special equipment, and therefore an alternative method of diagnosis which is less invasive, inexpensive and practical would be very beneficial to the equine industry. Healthy gastrointestinal mucosa is not permeable to sucrose and normally it is hydrolysed in the small intestine into monosaccharides which are then absorbed through the small intestine. When a horse has gastric ulcers, sucrose permeates through the damaged mucosa and can be detected in the blood. Based on this premise, sucrose serum concentration could be a useful alternative method to identify animals with gastric ulcers and to monitor the effect of therapy. Furthermore, the concentration of sucrose in serum may be correlated to ulcer severity. In order to investigate this hypothesis, a study is planned in which a large group of horses with and without naturally occurring gastric ulcers will undergo gastroscopy, followed by administration of sucrose and collection of sequential blood samples. Sucrose

concentration in blood will then be correlated to severity of gastric ulceration in order to determine if sucrose concentration in serum is a reliable marker of gastric ulceration.

To perform gastroscopy, the horses will need to be sedated. Detomidine and butorphanol is a common combination to sedate a horse. Detomidine is an alpha-2 adrenergic agonist and it produces changes in cardiovascular function (Tranquilli et al 2007). According to several studies it also has an effect on gastric emptying. After administration of detomidine, gastric motility was decreased. Similar effects have been found with other sedatives. (Vainionpää et al 2013, Elfenbein et al 2009, Merritt et al 1998, Sutton et al 2006.) Butorphanol is a synthetic agonist-antagonist opioid. Butorphanol has also been reported to have a delaying effect on gastrointestinal motility (Knych et al 2012, Sellon et al 2001.) If gastrointestinal motility is reduced, sucrose may stay longer in the stomach because of delayed gastric emptying, and that may have an effect on sucrose serum concentration when performing the sucrose permeability test on sedated horses. Conclusions drawn from the study about the reliability of the sucrose test to diagnose ulcers may not be comparable with a similar study performed on non-sedated horses. Therefore, it is the objective of this study to investigate the effect of sedation on sucrose permeability in the stomach.

2 LITERATURE REVIEW

2.1 Equine gastrointestinal anatomy and physiology

The gastrointestinal tract starts from the oral cavity and ends at the rectum. It is composed of five layers: mucosa, submucosa, circular muscle fibres, longitudinal muscle fibres and serosa. Sympathetic and parasympathetic branches relay neural signals from the central nervous system and influence gastrointestinal function. Peristalsis, secretion and blood flow are usually regulated locally. (Wong et al. 2011.) The enteric nervous system is independent of the central nervous system. It consists of

neurons in the gastrointestinal tract that control microcirculation, motility, exocrine and endocrine secretions. (Koenig & Cote 2006.)

Interstitial cells of Cajal (ICC) are pacemakers and mediators of certain forms of neurotransmission in the equine gastrointestinal tract. ICC take part in generating and maintaining normal gastrointestinal motility patterns. One of the most important properties is the generation of slow waves. Slow waves are continuous rhythmic pacemaker currents which take part in rate-limiting the peristaltic activity. ICC share common development origin with smooth muscle cells and are connected to them through low resistance electrical junctions. When current is generated in ICC, depolarization causes the activation of voltage-dependent Ca^{2+} channels in smooth muscle cells. Current is transmitted to smooth muscle cells and the potential of gastrointestinal contractions is set. Neural inputs can affect the smooth muscle cell response to slow waves. Excitatory neurotransmitters activate non-selective cation channels and cause the increase in effectiveness of slow waves to bring the smooth muscle membrane potential to threshold. Inhibitory neurotransmitters cause the opposite effect by activating K^+-channels. It has been proposed that ICC in the circular and longitudinal muscle layers of stomach would act as mechanoreceptors and be able to sense stretch of the muscle. (Fintl & Hudson 2010.)

For our study, the most important part of the gastrointestinal tract is the stomach. Anatomically it can be divided into the cardiac orifice, fundus, body, pyloric antrum and pylorus. It has a rich blood supply along the greater and lesser curvatures of the stomach. (Ellis 2011.) Stomach controls nutrient and drug delivery to the intestines. It grinds and mixes the content and releases it to the duodenum at a rate controlled duodeno-gastric reflexes. Gastric muscle contractions are two types: slow volume-reducing contractions of fundus that drive gastric emptying and peristaltic contraction waves of antrus that grind and mix the content of stomach. Basically fundus is a reservoir and supplier of chyme to the antrum where the antral contraction waves then break down and mix the chime. Pyloric sphincter controls the releasing rate of content to the duodenum. (Pal et al 2007.)

2.1.1 Measurement of gastrointestinal motility

Horses have different disease syndromes that have been associated with gastric emptying disorders. To learn more about this association, measurement of gastrointestinal motility has become necessary. Gastrointestinal motility is determined as the net movement of intraluminal contents. There are three basic parameters to measure gastrointestinal motility: myoelectric activity, mechanical activity and transit of intraluminal contents. These parameters do not always accurately correlate with each other. (Koenig & Cote 2006.) Nuclear scintigraphy is the most common method to measure gastrointestinal motility. In addition there are studies investigating orally administrated acetaminophen markers, lactose 13C-ureide breath test, the hydrogen breath test and wireless ambulatory capsules as possible measuring methods.

2.1.1.1 Nuclear scintigraphy

Nuclear scintigraphy is a common technique to evaluate gastric emptying. It is easy to perform, accurate, sensitive and quantitative. The procedure is performed in a special nuclear scintigraphy room. Horses are fasted to empty the stomach and then administered a radioactive isotope via nasogastric tube followed by serial imaging of the stomach region . Radioactive isotopes emit radiation that is captured by external detectors, gamma cameras, to form two-dimensional images. Images taken at different time points provide information about the location of the radioisotopes and consequently the rate of gastric emptying. (Lohmann et all 2000.) Although nuclear scintigraphy is very useful for measurement of gastrointestinal motility, it is not always possible to use. Nuclear scintigraphy is expensive and personnel need special training to work with it. Therefore alternative methods for the evaluation of gastric emptying have been studied.

2.1.1.2 Acetaminophen marker

Acetaminophen, or paracetamol is a mild analgesic drug that is readily absorbed in the small intestine but not in the stomach. Lohmann et al. (2000) demonstrated that absorption variables of orally administrated acetaminophen could be used as method to evaluate gastric emptying rate in horses. They found significant correlations with the values they gained from nuclear scintigraphy. This indicates that acetaminophen absorption may also be a valid method when evaluating gastric emptying rates in horses. Later on a similar of study was made by Lohmann et al (2002) in which gastric emptying was delayed using atropine sulfate, then determined with nuclear scintigraphy and absorption variables of orally administered acetaminophen. There was again significant correlation between the half-time of liquid-phase gastric emptying (T50) of both methods. In addition, Doherty et al (1998) had studied acetaminophen as a marker of gastric emptying and had published similar results.

2.1.1.3 Lactose 13C-ureide breath test and the hydrogen breath test

Undigested carbohydrate arrives in the caecum which starts the microbial H_2 production. H_2 diffuses into mucosal capillary blood and produces a detectable increase in end expiratory breath H_2 output. This is measured with the hydrogen breath test. Lactose ^{13}C-ureide (^{13}C-LU) is a glycosylureide compound, consisting of a sugar moiety (galactose-glucose) bound to a ^{13}C-urea molecule. The glucose-urea bond is cleaved by colonic microflora. C-urea is then released and hydrolyzed. The lactose ^{13}C-ureide breath test measures the rate of appearance of exhaled $^{13}CO_2$ after ingestion of the isotope. Sutton et al. (2011) compared the lactose ^{13}C-ureide breath test (LUBT) with the hydrogen breath test (H_2BT) for measuring the equine orocaecal transit time (OCTT). In the study they tested 8 horses with combined LUBT and H_2BT. Sequential end expiratory breath samples were analyzed and OCTT calculated. They found that there were peaks in expiratory $^{13}CO_2$ in all individuals after ingestion of the labeled test meal whereas H_2 expiration was not consistent. Their conclusion was that the LUBT would be more reliable than the H_2BT when measuring equine OCTT.

2.1.1.4 Wireless ambulatory capsules

Wireless ambulatory capsules are ingestible capsules used on humans to measure pressure, pH and temperature as it travels through the gastrointestinal tract to assess gastrointestinal motility. Stokes et al (2012) used wireless ambulatory capsules to determine gastric emptying time in ponies. WAC were administrated into the stomach. Data onluminal pH, temperature and pressure were collected by a receiver. Gastric emptying was presumed to have occurred when pH reached 8. Some of the ponies also went through the standard nuclear scintigraphy test and the results were compared with WAC gastric emptying time. In this study WAC provided non-invasive physiological information, but the results were variable. Gastric emptying time as determined by nuclear scintigraphy did not correlate significantly with gastric emptying time determined by WAC. The study showed that at the moment WAC is not a reliable tool for determination of gastric emptying in the horse.

2.1.2 Factors affecting gastrointestinal motility

While medicating the horse, we may also affect the gastrointestinal motility. Alternations on gastrointestinal motility may cause gastrointestinal disease e.g. ileus.Therefore the effect of medications on gastrointestinal motility has been widely studied in horses. Studies have mostly concentrated on the effects of sedation and analgesia.

2.1.2.1 Sedatives

The adverse effects of different sedatives on gastrointestinal motility of horses have been of interest to many researchers. Merritt et al (1998) studied the effects of xylazine, detomidine and a combination of xylazine and butorphanol on duodenal motility in 4

healthy horses. With all treatments, frequency of pressure peaks recorded from the gastric cannula were significantly reduced below their respective pretreatment values, but to variable degrees and durations. They found no significant difference between baseline values. Xylazine had the mildest effects that lasted 30 minutes after treatment. Detomidine had significant effects for 1 hour as well as the combination of xylazine and butorphanol. Merritt, Campbell-Thompson and Lowrey (1989) had earlier studied the effect of xylazine treatment on proximal gastrointestinal tract myoelectrical activity in 5 healthy horses. They found that xylazine resets the duodenal migrating motility complex in the horse, but causes no major disruption to the proximal gastrointestinal tract motility. Elfenbein et al. (2009) studied the effects of detomidine on visceral and somatic nociception and duodenal motility. Among other influences (decrease in nose-to-ground height, heart rate, skin temperature and respiratory rate), detomidine also significantly increased duodenal distension threshold and caused an immediate decrease in the amplitude of duodenal contractions for 50 minutes. Sutton et al. (2002) also found that detomidine has dose related inhibitory effects on gastric emptying. Detomidine prolonged both the gastric half-emptying time and duration of the lag phase. In the same study, xylazine also lengthened the duration of the lag phase, but the effect on the gastric half-emptying time was not significant. Doherty et al. (1999a) studied the effect of sedation on gastric emptying rate by monitoring serum concentrations of acetaminophen. In the study they used xylazine, butorphanol and acepromazine for sedation. After sedation the time to reach peak serum concentration increased significantly. Peak serum concentration decreased after xylazine was administrated and AUC decreased after acepromazine administration. The results indicate a delay in gastric emptying after sedation. In the study by Mitchell et al. (2005) the effect of xylazine was analyzed with ultrasonography. The main result was the effect of the feeding status before sedation with xylazine. Although gastrointestinal motility was decreased after administration of xylazine, horses that were fed had minimal changes in intestinal activity whereas changes with fasted horses were significant. Elfenbein et al (2011) studied the effect of ketamine as continuous rate infusion. Ketamine is a noncompetitive N-methyl-D-aspartate receptor antagonist often used as anesthetic. However, this study handled ketamine as an alternative analgetic. The result was that like other anesthetics, ketamine increased the gastrointestinal transit time and decreased fecal output.

2.1.2.2 Analgesics

Drugs with analgesic impact have effects on gastrointestinal motility in different ways. Widely used non-steroidal anti-inflammatory drugs (NSAID´s), can cause damage to the gastrointestinal tract (Videla 2009). However, it seems that NSAIDs don´t have an impact on gastrointestinal motility. There were only a few studies about NSAID´s and gastrointestinal motility and in those studies the research was concentrated on the effect of NSAIDs in combination with endotoxemia (Valk et al 1998b).

Opioid agonists are used for moderate to severe pain in horses especially when there is a need to avoid the adverse effects of NSAID´s on the gastrointestinal tract. Opioids stimulate receptors in the gastrointestinal tract that cause alterations in motility, secretion, absorption, and blood flow (De Luca & Coupar 2006.) Boscan et al (2006a) investigated the result of this stimulation using morphine. They found that morphine increased intestinal transit time and decreased secretion of water into feces. They also studied (2006b) if this effect could be override with opioid antagonist N-methylnaltrexone. The gastrointestinal effects of morphine were not significantly decreased by N-methylnaltrexone. Fugueiredo et al also (2012) studied the effects of morphine and got results indicating that morphine reduces gastrointestinal motility. Buprenorphine seem to have same kind of effects administrated intravenous or intramuscular route (Davis et al 2011).

2.1.2.3 Other drugs

Studies of the other drugs are mainly concentrated on treatment of gastrointestinal motility dysfunctions. Okamura et al. (2009) studied gastrointestinal prokinetic agents, mosapride, metoclopramide, cisapride and lidocaine that are used to treat decreased gastrointestinal motility. They found that mosapride, metoclopramide and cisapride all improved motility in the jejunum and mosapride also improved motility in the caecum.

Doherty et al (1999b), Valkt et al (1998a,b) and Meisler et al (2010) used serum concentrations of acetaminophen as a method to measure gastrointestinal motility when they studied the effect of drugs and endotoxin on gastric emptying. Doherty et al (1999b) divided horses in three groups: normal, fasted horses, horses given endotoxin intravenously and horses given metoclopramide and endotoxin intravenously. They compared serum acetaminophen concentration T_{max}, C_{max} and AUC among treatment groups. The study showed that endotoxin administration delayed gastric emptying and metoclopramide significantly ameliorated the effect. Valk et al studied the effect of cisapride (1998a) and phenylbutazone (1998b). In normal horses neither drug altered gastric emptying rates. However, when the drugs were separately combined with administration of endotoxin, cisapride and phenylbutazone attenuated the delay on gastric emptying caused by endotoxin. Meisler et al (2010) divided horses into two groups: horses given endotoxin and horses given endotoxin plus yohimbine, an alpha2 adrenergic receptor antagonist. The results were the same as Doherty et al (1996b) got with metoclopramide and Valk et al (1998) with cisapride. Yohimbine seems to prevent the delaying effect of endotoxin on gastric emptying. Doherty et al (1998) used acetaminophen as a liquid marker and found in their study that atropine delayed gastric emptying and erythromycin had no effect. Yohimbine increased absorption of acetaminophen. Maher et al (2008) studied the effect of ranitidine on gastric emptying. Ranitidine is a histamine type 2 receptor antagonist and it is used in treatment of gastric ulcers to inhibit gastric acid secretion. Gastric emptying was measured using acetaminophen absorption model. According to the results, ranitidine didn´t have any significant effect on gastric emptying in vivo. Neostigmine methylsulfate was found to delay gastric emptying of particulate markers in the study made by Adams and MacHarg (1985). Horses were given plastic markers. Horses which were given neostigmine methylsulfate had more markers in the stomach and caudal third of the small intestine compared to the control group.

2.1.2.4 Other

Only a few studies were found which investigated factors other than drugs affecting gastrointestinal motility. In a study made by Lammers et al (2005) gastric emptying was determined with serum acetaminophen concentration when horses had an indwelling nasogastric tube. No impact for indwelling nasogastric tube on gastric emptying was found in this study. However, Cruz et al (2006) studied the same subject and their results differed from the earlier study. According to their results, indwelling nasogastric tubes delayed gastric emptying.

Lorenzo-Figueras et al (2005) studied how the high-fat versus high-carbohydrate diet effects on gastric relaxation and emptying. Gastric emptying was measured with the ^{13}C-octanoic acid breath test and gastric tone by variations in volume of an intragastric bag. Gastric emptying rates didn´t differ significantly, though high-carbohydrate meals emptied initially more slowly.

2.2 Effects of sedation

In our study, horses were sedated in order to perform gastroscopy. Sedation on standing horses is also commonly preferred to general anesthesia when performing diagnostic and minor surgical procedures. General anesthesia is associated with a greater risk of mortality and morbidity in horses. General anesthesia is also expensive and requires expertise and appropriate facilities. (Johnston et al 2004.) Acepromazine and the alpha2 adrenoceptor agonists are commonly used for sedation in horses. The sedative effect of these individual agents can be accelerated with the simultaneous use of opioids. (Taylor et al 2013.) Usually a single bolus of an alpha2 agonist will last long enough to finish a short procedure. With longer procedures, a continuous rate infusion could be a better choice than repeated injections. (Haininsch 2001.) Sedatives are used to cause a relaxed state. Williams et al (2012) studied with EEG if the state is sleep-like. Six horses were administrated acepromazine, butorphanol, xylazine and detomidine, each drug at separate time points and the results were recorded on an EEG. The effects varied between different sedatives, but qualitatively EEG findings on sedated horses matched with findings during natural sleep. Xylazine and detomidine triggered sleep, but it was limited to slow wave sleep. Xylazine and detomidine also produced heart block and

startle responses similar to sleep. Acepromazine didn´t promote a relaxed state in each case.

2.2.1 Detomidine

To sedate horses in the study, we used the combination of detomidine and butorphanol intravenously. Detomidine is an alpha-2 adrenergic agonist. It is commonly used to provide sedation, analgesia and muscle relaxation in horses. Alpha-2 agonists bind to and stimulate alpha-2 adrenergic receptors in the central nervous system and periphery. The effect of detomidine is stronger and lasts longer than sedation and analgesia produced by xylazine. Detomidine allows evaluation and treatment of horses that would otherwise be hard to handle. (Daunt, Steffey 2002.)

Detomidine is administrated via intravenous, intramuscular or sublingual route. When administrated intravenously, sedation effect is quicker and with higher magnitude as well as other physiological and behavioral changes. (Mama et al. 2009.) In a study by l'Ami et al (2013) detomidine was given sublingually. According to their results, maximal ataxia was at 60 minutes and maximal sedation at 100 minutes. Also Gardner et al (2010) studied if detomidine could be used sublingually to cause an appropriate state to undergo veterinary and husbandry procedures under field conditions. Differences between detomidine and placebo was significant and the results promote the sublingual use of detomidine as one option. All parameters returned to baseline in 24 hours. Detomidine can also be administrated epidurally at the first intercoccygeal space. The drug is lipophilic and gets into the systemic circulation rapidly. Epidurally administrated detomidine provides a longer lasting sedation suitable for surgeries such as standing laparoscopic ovariectomy and cryptorchidectomy. (Virgin et al 2010.)

Detomidine is used alone and also in combination with butorphanol or other opiods to produce a more reliable sedation. Taylor et al (2013) studied if sedation requires both detomidine and buprenorphine. One group of horses was given detomidine and glucose intavenously, the other group detomidine and buprenorphine. According to the study, buprenorphine enhanced the sedation and the procedure was more likely to be

completed. There was no serious adverse effect marked; only ataxia was more severe and long-lasting with buprenorphine. Respiratory and cardiovascular effects were insignificant. Usually detomidine is given IV at doses ranging from 5 to 20 µg/kg or IM at doses ranging from 10 to 40 µg/kg. (Tranquilli et al 2007.)

Detomidine produces changes in cardiovascular function. Changes are dose-dependent. In conscious horses, cardiac output decreases by 40 % to 45 % and heart rate decreases by 30 % to 35 % after IV administration at doses of 10 to 20 microgram/kg. Detomidine administration effects very little on pulmonary function, but has greater effects on esophageal transit time and gastrointestinal motility. (Tranquilli et al 2007.) Detomidine´s side effects have been proved in several studies. According to study by Vainionpää et al (2013) and Elfenbein et al (2009), detomidine reduces heart rate significantly, causes heart blocks, reduces the gut sounds and prolongs defecation time after sedation. Yamashita et al (2000) compared cardiovascular effects of medetomidine, detomidine and xylazine. The effect on heart rate was insignificant with all drugs. Atrio-ventricular block was present with all drugs and it was most long-acting after administration of detomidine. All treatments produced hypertension as well as a decrease in cardio index and stroke volume. Medetomidine and detomidine decreased respiratory rate. All the effects were prolonged after administration of detomidine. Hyperglycemia has also been reported as one of the adverse effects (DiMaio Knych et al 2012). It has been studied, if the side effects could be eased when detomidine was combined with other drugs. Vaininopää et al (2013) used detomidine together with MK-467, a peripherally acting alpha-2-adrenoceptor antagonist that selectively targets alpha-2-adrenoceptors outside of the blood-brain barrier. According to their study, effects on intestinal motility and heart rate were significantly reduced when detomidine was used simultaneously with MK-467. DiMaio Knych et al (2012) gave horses yohimbine, a monoamine oxidase inhibitor that acts as an antagonist of alpha-2 adrenoceptors, 15 minutes after detomidine administration. Yohimbine reduced sedation, bradycardia, atrioventricular heart block and hyperglycemia caused by detomidine.

2.2.2 Butorphanol

Butorphanol is a synthetic agonist-antagonist opioid. It is a competitive μ-receptor antagonist, but exerts its analgesic action by acting as agonist at κ receptors. Butorphanol is been used with wide variety of veterinary species. Duration of analgesic effects depends on species, type and intensity of pain, dosage and route of administration. It is typically administrated via intravenous, the intramuscular or subcutaneous route. Butorphanol has very little effect on cardiopulmonary function. It does not induce histamine release when administrated intravenously. Butorphanol is often used in combination with other sedatives and tranquilizers, mainly with an alpha2-agonist or occasionally acepromazine. With horses it is administrated intravenously for standing procedures or prior to induction of general anesthesia. For visceral pain it has potent but short-lived analgesic effect. (Tranquilli et al 2007.) Sellon et al (2001) studied the effect of butorphanol when administrated by single intravenous injection or continuous intravenous infusion and continued with the topic later on comparing the pharmacokinetics of butorphanol after intramuscular and intravenous injection (2008). According their results, single intravenous injection caused adverse behavioral and gastrointestinal tract effects such as ataxia, decreased borborygmi, and decreased defecation that were minor or did not appear at all with continuous intravenous infusion. Although the adverse effects were minor with continuous intravenous infusion, the effect was prolonged and number of fecal piles passed during the 24-hour treatment period was significantly less in butorphanol treated horses when compared with controls. Intramuscular injection caused very little adverse affects, but to maintain target plasma butorphanol concentrations, injections every 3h would be necessary. Knych et al (2012) administrated butorphanol intravenously and reported adverse effects including decreased gastrointestinal motility and increase in heart rate.

2.3.1 Diagnosis of gastric ulcers

Gastric ulceration is typically diagnosed by nonspecific clinical signs and response to therapy (The Equine gastric ulcer council 1999). Clinical signs in adults are variable including weight loss, poor body condition, lack of appetite, mild or recurrent colic and loose faeces. Foals have more specific clinical signs such as poor body condition, less

time spent suckling, diarrhea, ptyalism, bruxism and intermittent colic. (Bell et al. 2007.)

For definitive antemortem diagnose of EGUS, gastroscopy is currently the only method. Horses are fasted for a minimum of 6 hours, pylorus visualized with 3-m endoscope and lesions scored according to severity. (Bell et al. 2007.) Two kinds of endoscopic equipments can be used: fibreoptic and video. To get a better view, the stomach is distended by insufflation with air. Additionally tap water is flushed through the biopsy channel to rinse gastric contents from the mucosa. (The Equine gastric ulcer council 1999.)

Because gastroscopy is not always possible, there are efforts to find alternatives for diagnostics of gastric ulcers. For instance, it has been reported that gastric ulcers could be evaluated by sucrose concentrations in urine after administration via nasogastric intubation. When gastric mucosa is healthy, sucrose is transported to the small intestine and hydrolyzed to glucose and fructose. Normally there are only trace amounts of sucrose in the urine. If the gastric mucosa is damaged, sucrose is able to penetrate the gastric wall and enter the circulation, filtered from the blood by the kidneys and finally concentrated and excreted in the urine. (O'Conner et al. 2004.) O 'Conner et al. (2004) studied the possibility to detect gastric ulcers in horses by evaluating urine sucrose concentrations. Horses were administered sucrose by nasogastric intubation and urine collected at 2 and 4 hours after intubation. Horses were also gastroscoped and gastric ulcers scored by severity with range 0 to 3. Horses were administrated omeprazole for 21 days and finally sucrose testing and endoscopy were repeated. They found in their study correlation with higher urine sucrose concentrations for horses with ulcer severity scores over 1. Urine sucrose concentrations and ulcer severity scores decreased after omeprazole treatment. They found the urine sucrose concentration to be a reliable but imperfect indicator of gastric squamous ulcers.

A faecal occult bloody test was found to be a possible method when diagnosing EGUS (Pellegrini 2005). Horses were euthanized and necroscopy was performed. A sample of feces was collected and examined with a correlated guaiac-based fecal occult test (gFOBT). 90 % of the horses with EGUS got positive predict value from gFOBT. Negative predict value was 17%. The study indicates that a horse with positive gFOBT

is likely to have gastric ulcer. Recent rectal biopsy, rectal examination or other rectal trauma may give a false positive test result.

Taharaguchi et al (2007) studied serum samples from foals. Objective was to find serum indicators of gastric ulcers. Gastric ulcers were detected endoscopically. They found serum α1-antirypsin, a plasma glycoprotein, in 44/47 foals with gastric ulcers and only 3/22 in healthy foals. α_1-antirypsin could be a protective response against proteolytic activities in damaged tissues, but more research is needed.

Hewetson et al (2006) studied the possibility to use sucrose concentration in blood as an assessment to detect gastric ulcers on horses. They suggested that increased sucrose concentration in blood would indicate gastrointestinal tract damage, for healthy gastrointestinal tract is not permeable for sucrose. Blood collection would be easier that urine collection, which is with horses technically demanding to perform. In the study horses were fasted and then administrated sucrose by nasogastric intubation. Blood samples were collected at 0, 15, 30, 45, 60, and 90 minutes. Horses were gastroscoped 4 hours later and gastric ulcers scored by severity with 4-point ulcer scoring system. They found that horses with moderate to severe gastric ulceration had a significant increase in serum sucrose concentration after administration of sucrose and after 45 minutes serum sucrose concentration was correlated with ulcer severity. Results of the study suggest that blood test may be a reliable screening test, though it would not replace gastroscopy. Blood test may help to identify animals with gastric ulcers and animals could then be gastroscoped. This would be cost saving for horse owners and help veterinarians that do not have access to gastroscopy. To get more information about this diagnostic possibility, a similar study design is planned with larger group of horses with and without naturally occurring gastric ulcers to determine if sucrose concentration in serum is a reliable marker of gastric ulceration. Horses will be sedated for gastroscopy and it is not clear if sedation has an effecton gastrointestinal motility. If sedation does alter gastrointestinal permeability, it may have an effect on sucrose serum concentrations when performing the sucrose permeability test on sedated horses. Therefore the objective of this study was to investigate the effect of sedation on the sucrose permeability curve.

3 MATERIALS AND METHODS

3.1 Subjects

Ten adult, clinically healthy horses were used in this study. The breeds comprised of eight Finnish horses and two warmbloods. Nine horses were geldings and one horse was a mare. Horses age ranged from 10.0 to 19.1 years (mean 14.0). The body weight ranged from 485 to 616 kg (mean 542 kg). The body condition was scored from 1 to 9, 1 being poor and 9 being extremely obese (Henneke et al. 1983). The mean body condition score was 6. A full clinical examination was performed on each horse. Horses were owned by the Equine college Ypäjä Finland and used for education of the students. The horses were housed separately in stalls. Free choice hay and water was provided for all horses, except for the fasting periods before both examinations (16 hours) and time before and during sucrose administration and blood collection. The protocol for this study was approved by the National Animal Experiment Board of Finland (Eläinkoelautakunta ELLA).

3.1 Experimental design

This study was a cross over study. 10 horses were sucrose tested without prior sedation and gastroscopy. After a washout period of 2 days, the same group of horses was sucrose tested again after sedation and gastroscopy, and the sucrose concentration in blood at each time point was compared.

3.2 Sucrose administration and collection of blood samples

Horses were fasted for 16 hours prior to both examinations. To ensure this, horses were fitted with muzzles. Fourteen gauge, 12,7 cm polyurethane catheters were placed in the left jugular vein of each horse and anchored with nylon stitches. Ten ml of blood was collected from the cannula and placed into vacuumed clot tubes. After blood collection the cannula was flushed with 10ml of heparin. For the first part of the experiment, sucrose was given to horses immediately after blood sample zero was collected. Horses were dosed with 500g sucrose as 10 % solution via nasogastric tube. Blood samples were then collected 45, 90, 135, 180 and 225 minutes after sucrose administration. After each blood collection the cannula was flushed with 10ml of heparin[a]. When the last blood collection was over, the catheter was removed and horses were given hay.

The second part of the experiment was performed two days later. After blood collection zero, horses were sedated with the combination of 0,3 ml detomidine hydrochloride[b] and 0,6ml butorphanol tartrate[c] intravenously and gastroscoped. To get the luminal surface of the stomach visible, the biopsy channel of the endoscope was used to distend the stomach by insufflation with air. Additionally tap water was flushed through the biopsy channel to rinse gastric contents from the mucosa. Gastroscopy was performed with a 3-m equine videoendoscope[d] and examinations were recorded. If found, gastric ulcers were located and scored using a published four-point gastric ulcer scoring system (Andrews et al 1999) (Table 1). When gastroscopy was finished, air was sucked through the biopsy channel in order to get the stomach deflated. Horses were dosed with 500g sucrose as a 10 % solution via nasogastric intubation immediately after gastroscopy had been performed. After that, blood samples were collected at 45, 90, 135, 180 and 225 minutes after sucrose administration. When the exam was over, the catheters were removed and the horses were given hay.

Table 1. Four-point gastric ulcer scoring system

Score	Description
0	Mucosal epithelium is intact; there may be hyperemia (reddening) or hyperkeratosis (yellow appearance to the squamous mucosa)
1	Small single or multiple ulcers
2	Large single or multiple ulcers
3	Extensive (often coalescing) ulcers with areas of apparent deep ulceration

3.3 Sample processing and analyses

Approximately 3 hours after the last collection, blood samples were centrifuged for 10 minutes at 3660g and the serum was frozen and stored at -80 ^{0}C . Serum samples were analyzed using a gas chromatography-flame ionization detection (GC-FID method. For GC-FID analysis samples were first thawed and mixed using a vortex and then two hundred µL of serum was added to an Eppendorf tube with 20 µL of internal standard (trehalose, 100 µg/ml). Zero point six mL acetonitrile-water (90:10) was added to the tubes and centrifuged for 10 min at 10 000 x g to precipitate proteins. The supernatant was set in a vacuum evaporator at 60 ^{0}C to dry. The residues were purged with nitrogen for 10 s and dissolved in 0.1 mL anhydrous pyridine and sonicated for 5 min. To convert sucrose and trehalose to their TMS derivative, 0,1 ml TMSI was added. Samples were capped tightly and heated at 100 ^{0}C for 1 hour. After reaction, the samples were centrifuged for 5 min at + 4 °C at 1711 x g and the aliquot of the mixture transferred to a autosampler vial. Analysis was done immediately.

An agilent 7890A instrument equipped with a flame ionization detector (FID) and Agilent 7683B autosampler (Agilent, China) was used for gas chromatography (GC). Single tapered glass liner fitted to an injector into a 2.5 m x 0.32 mm ID retention gap

(uncoated precolumn) was used to inject aliquots (1 µL) of the mixture in pulsed splitless mode at a pressure of 80 psi for 0.4 min. The retention gap and a 30 m x 0.320 mm I.D. fused-silica capillary column were attached, coated with 0.25 µm thickness HP-5 stationary phase (J&W Scientific, Folsom, CA, USA). Oven temperature was first held 95 °C for 2 min then increased at the rate of 30 °C/min till it was 205 °C where it was held at for 3 min and eventually oven temperature was increased to 250 °C at the rate of 1 °C/min. The temperatures for detector and injector were 300 °C and 230 °C. The purge flow rate was 60 ml/min and after 0.5 min the purge valve was turned on. Carrier gas for the mobile phase was helium at a constant flow rate of 4.8 ml/min. Helium (30 ml/min) was also used as make-up gas for the flame ionization detector. Auxiliary gases were hydrogen (45 ml/min) and synthetic air (400 ml/min). After every run, the column was backflushed at 300 °C for 10 void volumes.

3.4 Statistical analysis

The test hypothesis was that there is no difference between the two treatments (i.e. sedation and no sedation) at each time point. A Wilcoxon signed-rank test for non-parametric data was used to compare the cumulative amount of sucrose permeated at each time point with and without sedation. The p value and confidence intervals at each time point were then reported. Significance was set at $P<0.05$. The data was plotted on a line graph to create two sucrose permeability curves: with and without sedation.

4 RESULTS

4.1 Clinical examination, blood sample analysis and gastroscopy

A clinical examination was performed on all the horses. No abnormalities were found and the results for all the horses were normal. CBC and blood chemistry were mostly within reference range for all horses. Only a few clinically insignificant alterations occurred. Horse 003 had a slight increase in serum concentration of CK and AST (903 and 692 U/I, respectively) when compared to the reference interval in the laboratory (<350 and <604 U/I, respectively). Horse 010 had a slight increase in serum fibrinogen (4.6 g/l) when compared to the reference range (<4.0 g/l). Gastroscopy revealed endoscopically visible gastric ulceration in 8/10 (80 %) horses. Scores for these ulcers varied from 0 to 2.

4.2 Estimation of sucrose permeability

When horses were administered a 10 % sucrose solution via nasogastric tube, no adverse effects occurred and the solution was well tolerated among all the horses, both with or without sedation. Sucrose was detected in serum at time point 45 minutes and was still detectable at 225 minutes post-sucrose administration in all horses, sedated or not.

4.2.1 Non-sedated horses

When horses were not sedated, sucrose serum concentrations peaked for all horses between 45 and 90 minutes (7 horses peaked at 45 minutes and 3 horses at 90 minutes) and then gradually decreased. In the beginning, at time point zero, sucrose concentration was 250 pg/ µl in all horses. Mean concentrations of sucrose in serum peaked at 45 minutes and decreased gradually. Mean sucrose serum concentration was 2958,30 pg/µl when sucrose serum concentration peaked at 45 minutes and 1890 pg/ µl on last detection time point at 225 minutes.

4.2.2 Sedated horses

When horses were sedated, sucrose serum concentrations peaked for all horses between 45 and 180 minutes (4 horses peaked at 90 minutes, 3 horses at 135 minutes, 2 horses at 45 minutes and 1 horse at 180 minutes). Serum sucrose concentration decreased gradually, except with two horses where the sucrose serum concentration started to rise again at 180 minutes (one horse had peaked at 45 minutes and the other at 90 minutes) and was still increasing at 225 minutes and with one horse, which had two peaks in serum concentration, at 45 and 135 minutes. Mean concentration peaked at 90 minutes and decreased gradually. Mean sucrose serum was 1958,60 pg/µl at peaking time point and 1474,40 pg/µl at 225 minutes.

4.2.3 Group comparison

The cumulative increase in sucrose for non-sedated horses was significant ($p<0.05$) at all time points.

Table 2. Sucrose serum concentration [pg/µl] measured 0-225 minutes after administration of 10 % sucrose solution to clinically healthy, non-sedated horses.

	Horse No.	001	002	003	004	005	006	007	008	009	010	Mean	SD
Time after administration	0	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	0,00
	45	960,00	1313,00	4133,00	3247,00	2391,00	5987,00	1785,00	4142,00	4321,00	1304,00	2958,30	1667,66
	90	800,00	800,00	1872,00	3743,00	2026,00	5073,00	1490,00	4149,00	5280,00	1114,00	2634,70	1756,63
	135	800,00	800,00	2344,00	3539,00	1469,00	4523,00	1409,00	3695,00	4773,00	1184,00	2453,60	1546,84
	180	800,00	800,00	1557,00	2538,00	1295,00	3264,00	1086,00	3393,00	4543,00	1070,00	2034,60	1314,7
	225	800,00	800,00	1324,00	2865,00	1252,00	2878,00	964,00	2865,00	4290,00	862,00	1890,00	1231,77

Table 3. Sucrose serum concentration [pg/µl] measured 0-225 minutes after administration of 10 % sucrose solution to clinically healthy, sedated horses.

Time after administration	Horse No. 001	002	003	004	005	006	007	008	009	010	Mean	SD
0	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	250,00	0,00
45	800,00	1013,00	2760,00	1631,00	1010,00	1132,00	800,00	2726,00	4656,00	1588,00	1811,60	1233,68
90	1120,00	1295,00	2941,00	2142,00	1384,00	1798,00	800,00	3888,00	2723,00	1495,00	1958,60	963,932
135	1262,00	1787,00	3177,00	1719,00	1293,00	1360,00	910,00	2760,00	3093,00	1415,00	1877,60	824,386
180	897,00	1186,00	1488,00	1298,00	1360,00	1204,00	936,00	2228,00	3058,00	1721,00	1537,60	660,01
225	800,00	956,00	1882,00	1313,00	1934,00	1077,00	837,00	1881,00	2190,00	1874,00	1474,40	530,017

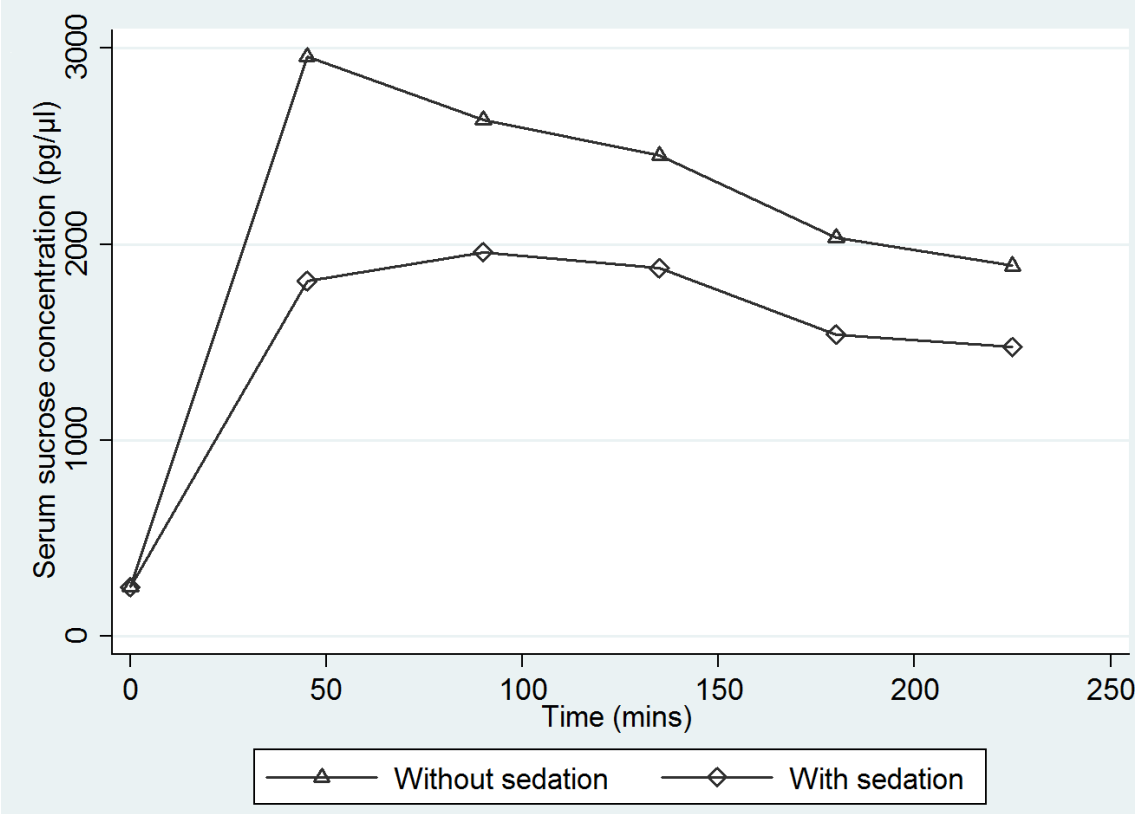

Figure 1. Sucrose serum concentrations in 10 clinically healthy horses with and without sedation after oral administration of 10 % sucrose solution.

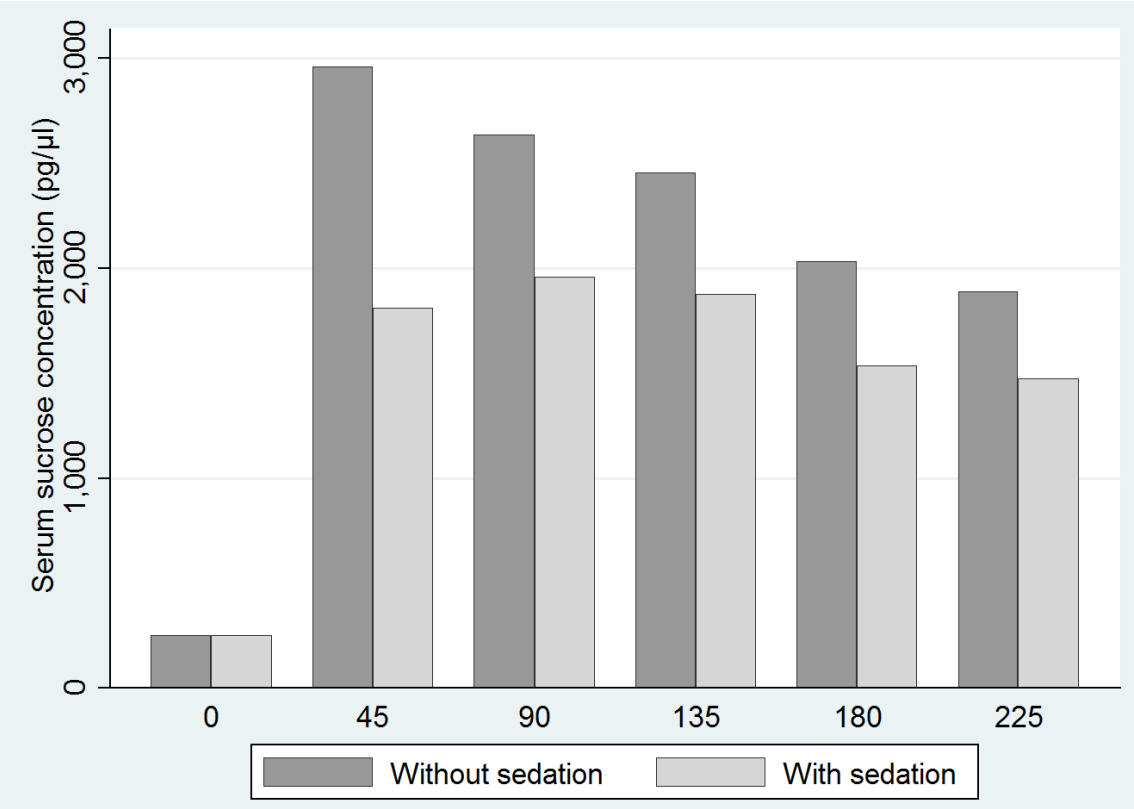

Figure 2. Sucrose serum concentrations in 10 clinically healthy horses with and without sedation after oral administration of 10 % sucrose solution.

5 DISCUSSION

5.1 Introduction

The objective of this study was to determine how sedation affects the sucrose permeation curve in horses with and without prior sedation. The study included two parts, first blood samples were collected at various time points from non-sedated horses and in the second part of the study from sedated horses. During the second part of the study the horses were also gastroscoped. At time point zero, all horses were administered sucrose through a nasogastric tube. Blood samples were then analyzed for

sucrose serum concentration. Using the sucrose serum concentration curve, sucrose permeability could then be compared with sedated and non-sedated horses to determine if sedation and /or gastroscopy affected sucrose permeation.

5.2 Clinical examination, blood results and gastroscopy

Study horses were clinically examined and blood samples taken for CBC and serum biochemistry. Results were normal. This ruled out basic illnesses that would effect on sucrose permeability or gastrointestinal motility. Horses were also gastroscoped in order to demonstrate a situation from real life. The majority of the horses (80 %) had evidence of mild gastric ulcers. Even though gastric ulcers may effect on sucrose permeability, this did not influence the results because the same horses were used as a control group.

5.3 Level of sedation

This study was performed to investigate the effect of sedation on sucrose concentration in blood. Sedatives used in this study were detomidine and butorphanol. This combination is often used for sedation on horses. When an alpha-2 adrenergic agonist and an opioid are combined, sedation is more reliable (Tranquilli et al 2007.) Detomidine and butorphanol were administrated intravenously which provides a potent and longer sedation compared to intravenous or sublingual route (Mama et al 2009.) The manufacturer recommends 0,1-0,3 ml/100g detomidine combined with 0,4-0,5 ml/100g butorphanol intravenously for sedation. The mean weight of the study horses was 542 kg. In the study we used 0,3 ml detomidine and 0,6 ml butorphanol to induce mild sedation. Horses needed to be able to walk from the stables to the examination room where gastroscopy was performed and back. The level of sedation was adequate to perform gastroscopy successfully. Study horses were used for education and were therefore used to being handled by strangers. In practice, some patients might need a stronger level of sedation. Sutton et al (2002) found in their study that the effect of

detomidine on gastric emptying time is dose dependent. In that case, different doses in the same horse could have an effect on gastric emptying and therefore on sucrose serum concentration. For this reason, if gastric ulcers were monitored by sucrose concentration in blood, it is recommended that the same dose of sedation is used each time.

5.4 Effect of sedation on sucrose permeation

Our hypothesis was that sedation slows down gastric motility. Many studies have come to the same conclusion. Merritt et al (1998) investigated detomidine and found significant effects on duodenal motility for up to 1 hour. Elfenbein et al (2009) found similar results, except that the effect lasted for 50 minutes. In our study the last blood sample was collected at 225 minutes and there was still a difference in serum sucrose concentration between sedated and non-sedated horses. Movements of the stomach transport the content of stomach to the small intestine. In the small intestine sucrose is hydrolyzed into glucose and fructose. When gastric motility slows down, sucrose is transferred to the intestines after longer period of time than normally. In that case sucrose would have more time to permeate through the stomach mucosa. According to our hypothesis, serum concentration would be higher with sedated horses than with non-sedated horses whose normal gastric motility would carry the sucrose faster to the intestines to be broken down. However, the results of our study show lower sucrose concentrations in blood for sedated horses. Although the shape of the curve stayed the same with sedated and non-sedated horses, the sucrose permeability values with sedated horses were lower in its entirety. Also sucrose serum concentration peaked later with sedated horses. Mean peak time point was 45 minutes with non-sedated and 90 minutes with sedated horses. This indicates that when horse is sedated, sucrose permeability is decreased and occurs more slowly than when they are not sedated. These results are contrary to our hypothesis.

One explanation could be the whole gastroscopy process that was only performed on sedated horses. The stomach was distended by insufflation of air in order to get the luminal surface of the stomach visible. After gastroscopy the stomach was deflated. It is

conceivable that the air insufflated is not completely deflated and the stomach stays more distended than normally. Sucrose lies in the bottom of the stomach and therefore only a limited area of gastric mucosa is exposed to sucrose. In contrast, horses that were not sedated and did not undergo gastroscopy would have very little air in the stomach, allowing for a extensive contact between the mucosa and the sucrose solution. If sucrose does not contact with the whole gastric mucosa, less amount of sucrose is absorbed in the stomach and larger amount of sucrose is hydrolyzed in the small testiness and is not detected in the blood samples. When a horse is not sedated, pleated stomach walls expose the gastric mucosa to the stomach content and more sucrose is absorbed before being transporting to the intestines. Sucrose serum concentration peaks earlier and therefore sucrose concentration in blood is higher.

5.5 Peak serum concentration

In our study sucrose serum peaked at different time points with some horses. This is not suprising as Lohmann et al (2000) demonstrated that there is variation in gastric emptying rates between individuals. In their study, they found that the rate of liquid phase emptying in normal horses varied from 27.9 to 87.3 minutes. However, variation between individuals was much wider with sedated horses. One explanation could be gastric ulcers. When gastric motility decreases, sucrose stays in the stomach for a longer period of time and the possibility to permeate into the blood via gastric ulcers increases. In the study by Hewetson et al (2006), horses with moderate to severe gastric ulcers had a clear increase in sucrose serum concentration. Horses with grade 3 gastric ulcers had a high peak of serum sucrose concentration at 45 minutes. In that study horses were not sedated. The effect of sedation on serum sucrose permeability with different grades of gastric ulcers might be interesting to study, but it would need a large number of horses. The other explanation for variation of peak points with sedated horses is that the effect of sedation is variable with individuals. The body weight ranged from 485 to 616 kg, but dose of the sedatives was the same. The concentration of the sedatives was different in horses. That could have effect on the gastric emptying time.

6 CONCLUSION

Our hypothesis was that sedation would affect gastrointestinal motility and with the result that sucrose serum concentration would be higher when horses were sedated. Our study indicates that it is the opposite. Non-sedated horses had higher sucrose serum concentrations the sedated horses. Sucrose serum concentration also peaked later with sedated horses. Nevertheless, the sucrose permeability curve was the same with both groups. That indicates we can rely on our results when making conclusions about sucrose test reliability by extrapolating the results from sedated horses to horses that undergo sucrose testing without sedation. Because of the differences in sucrose concentrations, cut-off values for sedated and non-sedated horses need to be considered. For sedated horses they need to lower than for horses without sedation. Cut-off values are needed when assessing gastric permeability to diagnose gastric ulcers. Defining these cut-off values would need a big group of horses. There is a planned study to investigate if the concentration of sucrose in serum may be correlated to ulcer severity. At the same time it would define cut-off values for severity of gastric ulcers. The results of our study are clear and confirm that conclusions drawn from the study about the reliability of the sucrose test to diagnose ulcers with sedated horses is comparable with a similar study performed on non-sedated horses.

7 FOOTNOTES

[a] Heparin, LEO Pharma A/S, Ballerup, Denmark

[b] Domosedan, Orion oyj, Espoo, Finland

[c] Butordol, Intervet international B.V, Boxmeer, Netherlands

[d] Olympus GIF-100, ~~Oly~~mpus America Inc, Melville, NY, USA

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Appendix I

STUDY DATA SHEET

Date: Identification number:

Body Condition:	Sex:		Weight: (kg)	
Breed:	Age:			
General status:		Locomotion:		
Behaviour:		Coat:		
Management :	Stable vs. Pasture ?		Chenge: yes / no	
Feed	What is fed and how much? How often		Chenge: yes / no	
De-Worming	Interval	Last de-wormed	What product was used:	
Drugs	Name	Amount	Start date?	End date?
Physical Examination	Resp. Rate/min:		CRT / sec:	
	Pulse rate / min:		MM colour:	
	Temperature:			
Gastrointestinal function	Teeth:		Abdominal auscultation:	
	Abdominal appearance:		LU	RU
			LL	RL

Gastronomy findings	

Ulcer Score	0	1	2	3	4	5

Defecation	Amount	Consistency	Last passed

Notes :

Time	Study Activity blood samples	Actual time and notes
T0	Place temporary catheter	
T0	blood sample	
T0	Dose horses with 500g sucrose via nasogastric tube	
T45	Blood sample	
T90	Blood sample	
T135	Blood sample	
T180	Blood sample	
T225	Blood sample	

Appendix II

Clinical examination results Wb= Warm blood Fh= Finnhorse

Horse No.	1	2	3	4	5	6	7	8	9	10
Sex	gelding	gelding	mare	gelding	gelding	gelding	gelding	gelding	gelding	gelding
Breed	Wb	Fh	Wb	Fh	Fh	Fh	Fh	Fh	Fh	Fh
Weight (kg)	570	590	515	616	558	514	550	530	485	495
Body condition	7/9	5/9	2/3	2/3	7/9	2/3	7/9	7/9	2/3	7/9
Pulse rate/min	40	38	38	38	40	30	33	32	48	30
CRT/sec	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
MM colour	pink	pale pink	pink	pale pink	pink	pale pink	pale pink	pale pink	pale pink	pale pink
Temperature (C°)	36.6	37	37.3	37.4	37.5	37.6	37.6	37.5	37.4	37.6
Abdominal	auscultation									
LU	+	++	++	+	+	++	++	++	++	++
LL	+	++	++	+	+	++	++	++	++	++
RU	+	++	++	+	+	++	++	++	++	++
RL	+	++	++	+	+	++	++	++	++	++

Appendix III

Biochemistry and complete blood count (CBC) results. ND, not determined

Horse No.	001	002	003	004	005	006	007	008	009	010
Cl (mmol/l)	98.4	94.1	96.2	96.1	97.2	100.4	98.4	100.8	99.0	98.0
K (mmol/l)	4.3	4.0	4.1	4.0	4.0	4.3	3.5	3.9	3.9	4.0
NA (mmol/l)	135	133	137	136	138	136	141	142	138	139
ASAT (U/l)	296	395	692	376	399	552	377	364	357	339
CK (U/l)	192	234	903	292	260	279	289	294	198	428
GT (U/l)	13	30	27	21	21	25	19	21	20	20
Mg (mmol/l)	0.81	0.71	0.87	0.79	0.75	0.80	0.88	0.81	083	0.74
SDP (U/l)	0.5	6.4	34.4	13.6	5.7	18.1	8.4	15.5	7.4	3.2
ALP (U/l)	283	415	527	376	313	367	306	592	316	549
Alb (g/l)	34.1	29.2	35.4	33.2	30.6	33.6	33.5	34.1	36.4	32.1
Bil. Tot (µmol/l)	29.3	23.6	23.8	27.1	20.0	19.3	23.3	29.2	27.9	24.1
Glu (mmol/l)	3.9	4.1	5.5	4.5	4.4	4.7	4.2	4.2	4.7	4.6
Ca (mmol/l)	3.22	3.13	3.18	3.27	3.13	3.29	3.33	3.36	3.30	3.26
Crea (µmol/l)	122	111	97	104	102	97	91	114	113	100
Prot (g/l)	60	69	61	70	70	67	73	74	59	68

Horse No.	001	002	003	004	005	006	007	008	009	010
FB (g/l)	4.4	3.6	3.6	4.3	3.3	4.4	4.0	4.4	3.7	4.6
WBC ($x10^9$/l)	6.31	7.38	4.20	7.29	4.64	5.01	9.52	7.19	5.92	7.25
NEU ($x10^9$/l)	4.14	5.06	2.26	4.38	2.47	3.28	5.95	4.45	3.95	4.39
LYM ($x10^9$/l)	1.69	1.55	1.12	1.74	1.75	1.26	2.94	1.88	1.55	2.08
MONO ($x10^9$/l)	0.310	0.370	0.209	0.638	0.189	0.278	0.418	0.337	0.224	0.251
EOS ($x10^9$/l)	0.130	0.358	0.495	0.483	0.204	0.165	0.146	0.498	0.147	0.516
BASO ($x10^9$/l)	0.040	0.036	0.014	0.053	0.022	0.021	0.059	0.023	0.054	0.015
RBC ($x10^{12}$/l)	8.01	6.63	7.04	7.93	6.67	7.28	7.79	7.30	9.30	6.82
HGB (g/l)	132	112	118	131	112	119	130	135	158	116
HCT (%)	38.2	30.7	33.6	36.7	33.6	33.2	37.1	38.2	44.2	32.8
MCV (fl)	47.7	46.3	47.8	46.2	47.0	45.6	47.6	52.4	47.5	48.1
MCH (pg)	16.5	16.8	16.8	16.5	16.8	16.3	16.6	18.5	17.0	17.0
MCHC (g/l)	346	363	352	358	359	358	349	352	357	353
RDW (%CV)	28.2	25.0	22.9	25.0	26.5	25.0	27.2	24.4	24.4	24.3
PLT ($x10^9$/l)	137	152	104	149	124	122	160	158	160	145
MPV (fl)	10.2	9.35	10.3	ND	6,90	7.03	6.45	10.3	7.14	8.50