CARDIOVASCULAR AND RESPIRATORY IMPLICATIONS OF CONCURRENT ADMINISTRATION OF PHENYLBUTAZONE AND ROMIFIDINE IN HEALTHY ADULT HORSES

By

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Submitted in fulfilment of the requirements for the degree of MSc in the Faculty of Veterinary Science, University of Pretoria

May 2009

This dissertation is dedicated to my wonderful husband
Werner Kruger

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ACKNOWLEDGEMENTS

I would like to thank the following people for their contributions to this project:

1. Prof. Frik Stegmann, my promoter, for his help during the trial.
2. Prof. Alan Guthrie for allowing me to use the research horses, for acting as co-promoter and for financial support of the project.
3. Dr Brighton Dzikiti for always listening and giving good advice.
4. Prof Piet Becker for help with the statistical analysis
5. Cliffie Matijane for his help with handling and looking after the horses.
6. Annicky Modirwa for performing blood-gas analyses. Neither rain, nor shine deterred you from immediate analysis of my samples. You're a star!
7. Shayna Basson and Theuns Brits for always being willing to lend a hand. I really appreciate you two.
8. My wonderful students who volunteered their time to entertain me and the horses. Tenaele, Bronwyn, Carolynne and Jean-Michel thank you very much for holding the horses.
9. Prof. Piet Stadler and the CACS department for financial support of the project.
10. RESCOM for their financial support.
11. Bayer, for donating the Phenylarthrite used in this trial.

ABSTRACT

Phenylbutazone is one of the most widely-used non-steroidal anti-inflammatory drugs in equine practice. According to literature, it does not have cardiorespiratory effects in healthy, standing horses, but anaesthesiologists at the Onderstepoort Veterinary Academic Hospital have experienced increased intra-operative haemodynamic instability associated with the use of the intravenous formulation directly prior to anaesthesia. Romifidine is widely used in equine practice as a sedative and adjunct to anaesthesia. It is known to have adverse cardiorespiratory effects, but these effects have only been characterised up to 2 hours after administration. The aim of this study was to characterise the cardiovascular and respiratory effects of phenylbutazone and romifidine individually over a 3-hour period and to establish whether there may be interactions between them.

A blinded crossover trial was performed in 5 horses. All horses were administered saline (Plac) IV, phenylbutazone 4.4 mg/kg IV (Pbz), romifidine 80µg/kg IV (Rom) and a combination of phenylbutazone 4.4 mg/kg IV and romifidine 80 µg/kg IV (Pbz_Rom), with at least one week washout period between treatments. Heart rate, respiratory rate, arterial pressures and central venous pressure were recorded directly prior to drug administration and every 5 minutes thereafter for a 3-hour period. Arterial and venous blood-gas and electrolyte values were assessed directly prior to and at 10, 60, 120 and 180 minutes after drug administration. Electrocardiographic abnormalities, the level of sedation and frequency of urination were recorded. Data was assessed using ANOVA for the maximum change from baseline during the first 30 minutes and changes over time within groups were assessed descriptively.

There were statistically significant differences between treatment groups for maximum change in central venous pressure in the first 30 minutes after treatment. All groups differed significantly from all other groups, except Rom and Pbz_Rom.

Descriptive findings included a transient increase in heart rate in the Pbz group as well as a transient decrease in arterial pressures. In the Rom group, there was an initial increase in central venous pressure, peaking at 5 min, followed by a decrease to a minimum at 105 min, where after it slowly started to recover. Systolic and mean arterial pressures were decreased, reaching a minimum at 105 min. Diastolic arterial pressure showed an initial increase to a maximum at 5 min, followed by a decrease to a minimum at 105 min. All these parameters as well as heart rate, respiratory rate and PvO_2 were still decreased at 175 min. Serum Ca^{2+} and K^+ decreased during the observational period and PCO_2 and HCO_3^- increased. Pbz_Rom showed similar trends to Rom, but the depressant effect on heart rate, arterial pressure and central venous pressure was less severe, and the decrease in serum Ca^{2+} and K^+ more severe than Pbz or Rom.

We conclude that romifidine's depressant cardiorespiratory effects are prolonged beyond 175 min. No interaction between phenylbutazone and romifidine was found. This is a pilot study and larger studies are required to establish whether the observed changes in this trial are statistically significant.

CHAPTER 1: GENERAL INTRODUCTION

Two healthy adult horses at the Onderstepoort Veterinary Academic Hospital (OVAH), who received routine doses of intravenous (IV) phenylbutazone, demonstrated adverse effects. The first horse was being prepared for standing castration when the incident occurred. Phenylbutazone (2.2 mg/kg) had been injected slowly IV 15 min after sedation with detomidine (10 µg/kg). Approximately one minute after phenylbutazone administration, the horse collapsed with atrial fibrillation, which was confirmed using electrocardiography. This horse was resuscitated with cardiac massage and adrenalin. The second case, a healthy horse that had been premedicated with romifidine and phenylbutazone, developed cardiac arrest during anaesthetic induction with guaifenesin and ketamine. Resuscitation was not successful.

To our knowledge, there have been no reports of either interactions between these drugs, or severe short-term cardiovascular and respiratory side effects of phenylbutazone alone in healthy, standing horses. Romifidine and phenylbutazone are commonly used alone and in combination at the OVAH. Romifidine was chosen over detomidine for this study, due to it being used for premedication with much greater frequency in the OVAH.

The objectives of this trial were:

1. To determine the effect of phenylbutazone on the cardiovascular and respiratory systems when administered as a bolus at 4.4 mg/kg IV.

2. To characterize the cardiovascular and respiratory effects of romifidine at 80 µg/kg IV over a 3 hour period.
3. To determine whether combined administration of phenylbutazone and romifidine altered their individual cardiorespiratory effects.

CHAPTER 2: LITERATURE REVIEW

PHENYLBUTAZONE

Introduction

Phenylbutazone (1,2-diphenyl-3,5-diketo-4-n-butyl-pyrazolidine) is a non-steroidal anti-inflammatory drug (NSAID) that is commonly used peri-operatively and to treat acute and chronic musculoskeletal pain in horses (Lees 2003, Moses, Bertone 2002, Doucet et al. 2008). Like other NSAIDs, it has anti-inflammatory, analgesic and anti-pyretic properties.

Its most important mechanism of action is believed to be non-selective inhibition of enzymatic conversion of arachidonic acid to prostaglandin and thromboxane by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Lees 2003, Moses, Bertone 2002, Doucet et al. 2008). In addition to the inhibition of COX, other mechanisms for this class are being explored (Lees 2003, Doucet et al. 2008, Clark, Clark 1999, Rainsford 2007). The molecular mechanism of COX inhibition has been reviewed by Marnett (2002) (Marnett 2002).

Phenylbutazone is the most commonly used NSAID in horses despite its narrow margin of safety, which is due to inhibition of both COX-1 and COX-2 (Doucet et al. 2008). It also has the most severe side-effects when compared to other commonly-used NSAIDs (MacAllister et al. 1993).

Eicosanoids and Cyclooxygenase

Eicosanoids are metabolites of arachidonic acid that are produced in almost all cells and include prostaglandins, prostacyclin, thromboxane A_2, leukotrienes, lipoxins and hepoxylins. These compounds are not stored, but are continually produced. Production is limited by the availability of substrate, which is the amount of arachidonic acid liberated by phospholipase A_2. The key regulatory enzymes for the conversion of arachidonic acid to prostaglandin and thromboxane are COX-1 and COX-2. These enzymes act through identical pathways. NSAIDs such as phenylbutazone inhibit both forms of COX (but not lipoxygenase) (Moses, Bertone 2002, Dubois et al. 1998, Goodman et al. 2006).

Under normal physiological (basal) conditions eicosanoids regulate critical physiological responses to maintain homeostasis of e.g. blood vessel tone, haemostasis, bone metabolism, nerve growth and development, kidney function, gastrointestinal secretion etc. For this reason, COX-1 is found in nearly all tissues under basal conditions, where its major function is to provide prostaglandin precursors for homeostatic regulation. There are low or undetectable levels of COX-2 in most tissues under basal conditions. Its production is massively up-regulated in inflammatory and some neoplastic conditions but it has now also been found to have an important role in some basal functions (Dubois et al. 1998, Goodman et al. 2006).

Most of the side-effects of NSAIDs are thought to occur as a result of COX-1 inhibition, which interferes with the homeostatic maintenance functions of prostaglandins and thromboxane (Rainsford 2007, Dubois et al. 1998).

Toxicity

The inhibition of prostaglandin production by NSAIDs contributes to gastrointestinal ulceration, colitis, decreased renal blood flow (which may lead to medullary ischemia and renal papillary necrosis) and hyperkalemia (through the suppression of renin and aldosterone secretion) (Moses, Bertone 2002, Plumb 2005). However, various authors have reported a variety of toxic and enzyme-inhibitory effects that occur through non-prostaglandin mediated pathways (Meschter et al. 1990). Non-steroidal anti-inflammatory drugs may also affect proteoglycan synthesis, synovial membrane metabolism as well as osteoblastic and osteoclastic metabolism (Moses, Bertone 2002, Rainsford 2007). Blood dyscrasias, bronchoconstriction and premature closure of the ductus arteriosus have been reported. Intramuscular or extravascular phenylbutazone injections may cause cellulitis, thrombophlebitis or tissue necrosis (Moses, Bertone 2002, Plumb 2005).

In humans phenylbutazone is rarely used nowadays as it is known to cause gastrointestinal ulceration, sodium chloride and water retention, blood dyscrasias, lymphadenopathy, agranulocytosis, hepatotoxicity, nephrotoxicity and severe hypersensitivity reactions such as asthma and systemic lupus erythematosus (Biscarini 2000). Headache, dizziness, vertigo and psychomotor reactions during driving have also been reported (Biscarini 2000).

Interactions

These are thought to occur as a result of one of the following mechanisms (Plumb 2005, Biscarini 2000):

1. Protein displacement (phenylbutazone is >99% protein bound in horses)

2. Interference with tubular excretion (penicillins and oral hypoglycaemic drugs)
3. Induction of microsomal liver enzymes
4. The side-effects of NSAIDs may affect the effects of other drugs (e.g. the effect of antihypertensive agents may be nullified due to salt and water retention).

Cardiovascular effects

Historically, phenylbutazone was thought to have no direct effects on the heart and peripheral vessels (Jeffcott, Colles 1977). A minor, but significant increase in heart rate following slow IV administration of NSAIDs under halothane anaesthesia has been reported in horses (Brouwer 1987). One study in standing and running horses reports that phenylbutazone causes no detectable change in systemic haemodynamic variables (Hinchcliff, McKeever & Muir 1994). There is some controversy in literature regarding its effect on exercising horses. One study reports an increase in heart rate and right atrial pressures during exertion (Mitten, Hinchcliff & Pate 1996), and another no significant difference in either heart rate, right atrial or pulmonary vascular pressures (Manohar et al. 1996).

ROMIFIDINE

Introduction

Romifidine, an α_2–selective adrenergic agonist, is a potent sedative-hypnotic. The α_2 adrenergic agonists were originally developed as anti-hypertensives in man (Miller 2000). They are frequently used in equine practice for chemical restraint, analgesia and as part of balanced anaesthetic protocols (England, Clarke 1996).

In addition to romifidine's effects via adrenergic receptors, its imidazole ring enables it to interact with non-adrenergic imidazoline binding sites (Faber, Chambers & Evans 1998), which has synergistic central inhibitory effects with α_2 adrenergic receptors (Reis, Piletz 1997, Bruban et al. 2002).

Adrenergic receptors and homeostasis

There are nine distinct subtypes of adrenergic receptors (α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3) the majority of which are still under investigation for precise physiologic function and therapeutic potential (Philipp, Brede & Hein 2002). The exact effect of an α_2-selective adrenergic agonist binding to its receptor will vary according to receptor subtype and location. These receptors are located pre-, post-, and extrasynaptically in the cetral nervous system and peripherally in nerves, blood vessels and organs (Murrell, Hellebrekers 2005). Sympathetic neurotransmitters (such as norepinephrine) regulate autonomic sympathetic physiological processes through transmembrane receptors, which open or close ion channels and regulate the production of second messengers via G-protein mediated pathways. Examples of mechanisms include the inhibition of adenylyl cyclase (leading to decreased cAMP production), opening of K^+ channels (leading to efflux of K^+ from cells and membrane hyperpolarisation), inhibition of voltage gated Ca^{2+} channels (leading to inhibition of neurotransmitter release), acceleration of Na^+/H^+ exchange, stimulation of phospholipase C activity, arachidonic acid mobilization by phospholipase A_2 etc (Goodman et al. 2006, Murrell, Hellebrekers 2005).

In order to maintain autonomic homeostasis, there is continual basal activity of both the sympathetic and parasympathetic systems. This is referred to as sympathetic / parasympathetic tone (Guyton 1991). Under basal conditions, post-synaptic α_2 adrenergic receptors have physiologic functions in the liver, pancreas, platelets, kidney, adipose tissue and eye, while pre-synaptic α_2 receptor activation leads to a decrease in noradrenaline release in the central nervous system (thereby decreasing sympathetic tone) (Murrell, Hellebrekers 2005). Non-adrenergic systems interact with the adrenergic nervous system through heteroreceptors on sympathetic nerve varicosities. These receptors inhibit sympathetic neurotransmitter release and include muscarinic, serotonin, PGE_2, histamine, enkephalin and dopamine receptors (Goodman et al. 2006).

When discussing adrenergic receptor specificity of drugs it is important to note that specificity is only relative. Romifidine may have greater affinity for α_2 receptors, but that doesn't mean that it has no affinity for α_1 receptors.

One of the key functions of the auonomic system is the regulation of blood pressure. Adrenergic receptors in nerve terminals (centrally and peripherally), smooth muscle cells and endothelial cells (which are now known to possess at least five different adrenoceptor subtypes) are fundimental to the control of peripheral resistance (or vascular tone). Guimaraes and Moura (2001) present a detailed discussion of the present knowledge of vascular adrenoceptors (Guimaraes, Moura 2001). By acting on these receptors, adrenergic agonists will necessarily have profound effects on blood pressure and vascular tone.

Cardiovascular and respiratory effects

Cardiovascular and respiratory effects of Romifidine in healthy, standing horses includes: bradycardia, hypertension, increased systemic vascular resistance, hypotension, increased central venous pressure, heart blocks, decreased stroke volume, increased frequency of valvular regurgitation, decreased respiratory rate and interestingly an increase in the arterial and venous bicarbonate concentration and actual base excess (Browning, Collins 1994, Buhl et al. 2007, Clarke, England & Goossens 1991, Freeman et al. 2002, Gasthuys et al. 1990, Hamm, Turchi & Jochle 1995). Effects vary in onset and duration and are not always dose-dependent. Cardiovascular depression is prolonged, persisting even after clinical parameters such as heart rate have returned to normal (Freeman et al. 2002).

Interactions

Severe interactions between the α_2 adrenergic agonist detomidine and IV sulphonamide antibiotic (another highly protein-bound drug) injection have been reported in horses. Severe dysrhythmias, hypotension, apnoea and death have been reported (Dick, White 1987, Taylor et al. 1988).

COMBINED USE OF NSAIDS AND α_2 ADENERGIC AGONISTS

These drug classes have been used together (phenylbutazone and xylazine (Raekallio, Taylor & Bennett 1997), phenylbutazone and detomidine (Johnson et al. 1993)) by other authors, but never for the purpose of investigating adverse interactions. No adverse cardiovascular and respiratory effects specifically related to the use of these drugs were reported in these studies, but there is controversy in the literature regarding the anaesthetic recovery scores of horses that received phenylbytazone peri-

operatively. One author reported that the anaesthetic recovery score was significantly better in the placebo group than the phenylbutazone group (Raekallio, Taylor & Bennett 1997), while another had the impression that recoveries of horses that received phenylbutazone intra-operatively were smoother, though this clinical impression could not be substantiated statistically (Brouwer 1987). The former proposes an interaction between phenylbutazone and the protein-bound anaesthetic agents as a possible explanation for the difference in recovery scores (Raekallio, Taylor & Bennett 1997), but it is of interest for the purpose of this study that an α_2 adrenergic agonist was used in the anaesthetic protocol of the study that reports poorer recovery scores and not in the study reporting smoother recoveries.

There is a rational physiological basis for the possibility of an interaction between these drugs, since both are involved in homeostatic regulation. Examples of physiological overlap are:

- Adrenergic receptors stimulate phospholipase-A_2, leading to prostaglandin and leukotriene production via cyclooxygenase and lipoxygenase (Goodman et al. 2006). Phenylbutazone inhibits cyclooxygenase, which may cause shunting of substrate to the lipoxygenase pathway.
- PGE_2 inhibits sympathetic outflow by binding to heteroreceptors on nerve fibres. Phenylbutazone decreases prostaglandin production, which would increase sympathetic tone in these nerve fibres. The α_2 adrenergic agonists inhibit sympathetic outflow by decreasing the amount of norepinephrine released by neurons.

CONCLUSION

Phenylbutazone is the most commonly used non-steroidal anti-inflammatory drug in equine practice. Its precise molecular mechanism of action is poorly described. Phenylbutazone is known to interact with various drugs mostly as a result of protein binding. Evidence of significant cardiovascular and respiratory effects in healthy, standing horses is lacking.

Romifidine is an α_2 adrenergic agonist with significant cardiovascular and respiratory depressant effects. These effects have been characterized for two hours after administration. Clinical responses to α_2 adrenergic agonists are the result of complex interactions between various role-players in the autonomic adrenergic system.

There are no reports of specific negative interactions between phenylbutazone and romifidine.

CHAPTER 3:

CARDIOVASCULAR AND RESPIRATORY IMPLICATIONS OF CONCURRENT ADMINISTRATION OF PHENYLBUTAZONE AND ROMIFIDINE IN HEALTHY ADULT HORSES

3.1 INTRODUCTION

Phenylbutazone and romifidine are widely used in equine veterinary practice. The adverse events experienced at the OVAH, as well as some anaesthesiologists at the faculty's perception that horses receiving intravenous phenylbutazone directly prior to surgery experience more homeostatic instability during anaesthesia prompted this investigation.

Horses are at an increased risk for anaesthetic-related death compared to other species (Johnston et al. 2002, Johnston 2005). It is important for anaesthesiologists to be aware of the full extent and duration of each pre-operatively administered drug's effect in order to design the optimal anaesthetic protocol for each patient.

Romifidine is commonly used in anaesthetic protocols, both as pre-medicant (Johnston et al. 2002) and as a sedative in the recovery period. Romifidine's cardiorespiratory effects are severe and prolonged, leading to reports of residual sedation after anaesthesia when romifidine is used as premedication (Diamond et al. 1993). Despite this and the epidemiologic finding that its use is associated with an increased risk of anaesthetic death (Johnston et al. 2002), romifidine's cardiorespiratory effects have not been characterized beyond 2 hours after administration (Freeman et al. 2002).

Phenylbutazone is also used extensively peri-operatively in order to decrease post-operative pain and inflammation. It has been shown (in dogs) that pre-surgical NSAIDs are more effective than post-operative NSAIDs in reducing post-operative pain (Moses, Bertone 2002). Phenylbutazone is believed to be devoid of cardiovascular and respiratory effects in healthy, resting horses (Hinchcliff, McKeever & Muir 1994), but this finding is contrary to the experience of some of the OVAH clinicians.

The aim of this study is to characterize the cardiorespiratory effects of phenylbutazone and romifidine over a 3-hour period and to determine whether an interaction between them might have caused the adverse effects experienced in the reported incidents with two horses at the OVAH.

The hypotheses are:

1. Phenylbutazone has cardiorespiratory effects in the standing horse.
2. Romifidine has cardiorespiratory effects that are prolonged.
3. There is an interaction between phenylbutazone and romifidine that has cardiovascular or respiratory effects.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Animals

Seven healthy horses aged 4-17 years and weighing between 380kg and 500kg were allocated to this study from research populations at the Equine Research Centre (ERC) and the OVAH. Due to unforeseen circumstances, two of these horses were withdrawn from the study.

Horses were managed according to the Standard Operating Procedure of the ERC for the duration of the study. The support-staff of the ERC was responsible for the daily feeding, turnout and care of the experimental animals. For ease of arterial blood sampling and pressure monitoring, subcutaneous transposition of a carotid artery (Tavernor 1969) was performed on each of these horses one and a half months prior to the commencement of the trial. See Appendix B for details of adverse and unforeseen events occurring in the research population.

Horses were judged to be healthy on the basis of physical examination (temperature, pulse, respiratory rate, mucous membrane colour and capillary refill time, cardiac auscultation, electrocardiogram (ECG), thoracic auscultation and abdominal auscultation), complete blood count, clinical chemistry (total serum protein, albumin, globulin) and complete echocadiographic examination.

On each trial day, the study subjects were weighed and examined physically. Food and water was withheld for 30 min prior to treatment administration and during the setup and monitoring phases. The animals were restrained in stocks during

preparation before monitoring commenced and for the attachment of monitoring equipment, as well as for the duration of each 3-hour monitoring period. On two occasions a horse became unmanageable in the stocks during the three-hour monitoring period and hay was brought in to distract it until it calmed down.

3.2.2 Experimental Design

This study is a four period, four-treatment, blinded, randomised, crossover trial, with at least one-week washout period between treatments. The four treatments are:

1. Phenylbutazone (Phenylatrhrite, Bayer (Pty) Ltd.) at 4.4 mg/kg IV (Plumb 2005) injected over a period of 60 sec. (Pbz group)
2. Romifidine (Sedivet, Boehringer Ingelheim) at 80 µg/kg IV (Clarke, England & Goossens 1991, Clarke, England & Goossens 1991) injected over a period of 60 sec. (Rom group)
3. Phenylbutazone at 4.4 mg/kg IV and romifidine at 80 µg/kg IV injected over a period of 60 sec. (Pbz_Rom group)
4. Placebo (saline) IV injected over a period of 60 sec. (Plac group)

All treatments were administered directly after baseline values were recorded. The treatment order for each horse was randomised and the person collecting the data was blinded. The study protocol was approved by the Research and Ethics Committee of the Faculty of Veterinary Science, University of Pretoria, South Africa.

3.2.3 Instrumentation

A 12 gauge (Branule MT®) catheter was placed in the left jugular vein. Medical-grade polythene tubing was passed through the catheter and into the intra-thoracic cranial vena cava. This tube was connected to a pressure transducer and the Datex

Ohmeda Cardiocap 5 monitor and its position verified by waveform tracing. Once the tube was in the correct place, the catheter was removed from the vein and the tube sutured in place. Central venous pressure measurements were made and blood for blood-gas analysis was collected through this tube.

A 20 Ga, 8cm arterial catheter (Arrow International Inc.) was placed in the superficial carotid artery. The catheter was connected to a 3-way stopcock of which one arm was connected to an invasive blood pressure monitor (Datex Ohmeda) and the other used for arterial blood sampling. Prior to taking each measurement of arterial or central venous blood pressure, the monitor was zeroed with the transducer placed at the level of the left atrium. A three lead ECG was connected to the same monitor in a y- lead system.

3.2.4 Measurements

After completion of the instrumentation, each animal was allowed to settle for a few minutes before baseline cardiovascular and respiratory variables were recorded (t_o). The following parameters were recorded by the investigator at 5 min intervals:

- Heart Rate
- Systolic arterial pressure (SAP), mean arterial pressure (MAP) and diastolic arterial blood pressure (DAP)
- Central Venous Pressure (CVP)
- Respiratory Rate

Any ECG abnormalities seen on the monitor were recorded, as well as any adverse events and frequency of urination.

The level of sedation was recorded based on the 'ataxia' and 'response to stimuli' scales used by previous authors (England, Clarke & Goossens 1992). Parameters were recorded at 15 min intervals until the animal was found to be devoid of measured sedative effects for two consecutive measurements.

Arterial and central venous blood gas samples were taken at $t_{0,\ 10,\ 60,\ 120\ and\ 180}$. These samples were taken immediately on ice to the clinical pathology laboratory where they were analyzed within 10 min of collection. Parameters that were measured included pH, PO_2, PCO_2, Na^+, K^+, Ca^{++}, HCO_3^- and Base Excess (BE).

3.2.5 Data Analysis

The data was analysed using STATA^{TM} Statistical software: Release 10. Treatments (Plac, Rom, Pbz, Pbz_Rom) were compared with respect to maximum change from baseline at 30 minutes post-injection for clinical parameters (heart rate, respiratory rate, arterial pressures and central venous pressure). This was calculated as follows: {(maximum or minimum in the first 30 minutes (whichever was deviated further from baseline)} minus {baseline value}. An appropriate ANOVA was used to determine between-treatment differences from this crossover study with respect to the maximum change in the first 30 minutes after injection, with factors treatment, period and carry-over effect. Testing was done at the 0.05 level of significance. When significant treatment differences were found, Fisher's Least Significant Differences were calculated. Clinical and blood-gas data within each treatment could not be analyzed with repeated measures analysis of variance due to the small sample size, i.e. observation vector exceeded sample size. Hence, for both clinical and blood-gas parameters, progression within treatments were assessed for trend in an exploratory way only.

3.3. RESULTS

Five horses received all four treatments at random, with a minimum of one week washout period between drug administrations. Cardiovascular and respiratory results are summarised in tables 1 - 8 and figures 2.1 - 2.6. Blood-gas and electrolyte data is summarised in tables 9.1 - 16.2 and figures 3.1 - 3.16

BETWEEN-TREATMENT DIFFERENCES

Clinical data (Tables 1 & 2)

There were no statistically significant treatment effects for the maximum change in heart rate, respiratory rate, systolic arterial pressure, diastolic arterial pressure, or mean arterial pressure over the first 30 minutes.

For maximum change from baseline for central venous pressures in the first 30 minutes, treatments differed significantly ($p<0.000$). All treatments differed statistically significantly from all other treatments, except for Rom and Pbz_Rom, which did not differ significantly.

Specifically, Pbz showed a significantly greater change than Plac ($p=0.0013$), change in Rom was significantly greater than Plac ($p=0.0001$), change in Pbz was significantly greater than Plac ($p=0.0000$), change in Rom was significantly greater than Pbz ($p=0.0000$), and change in Pbz_Rom was significantly greater than Pbz ($p=0.0000$).

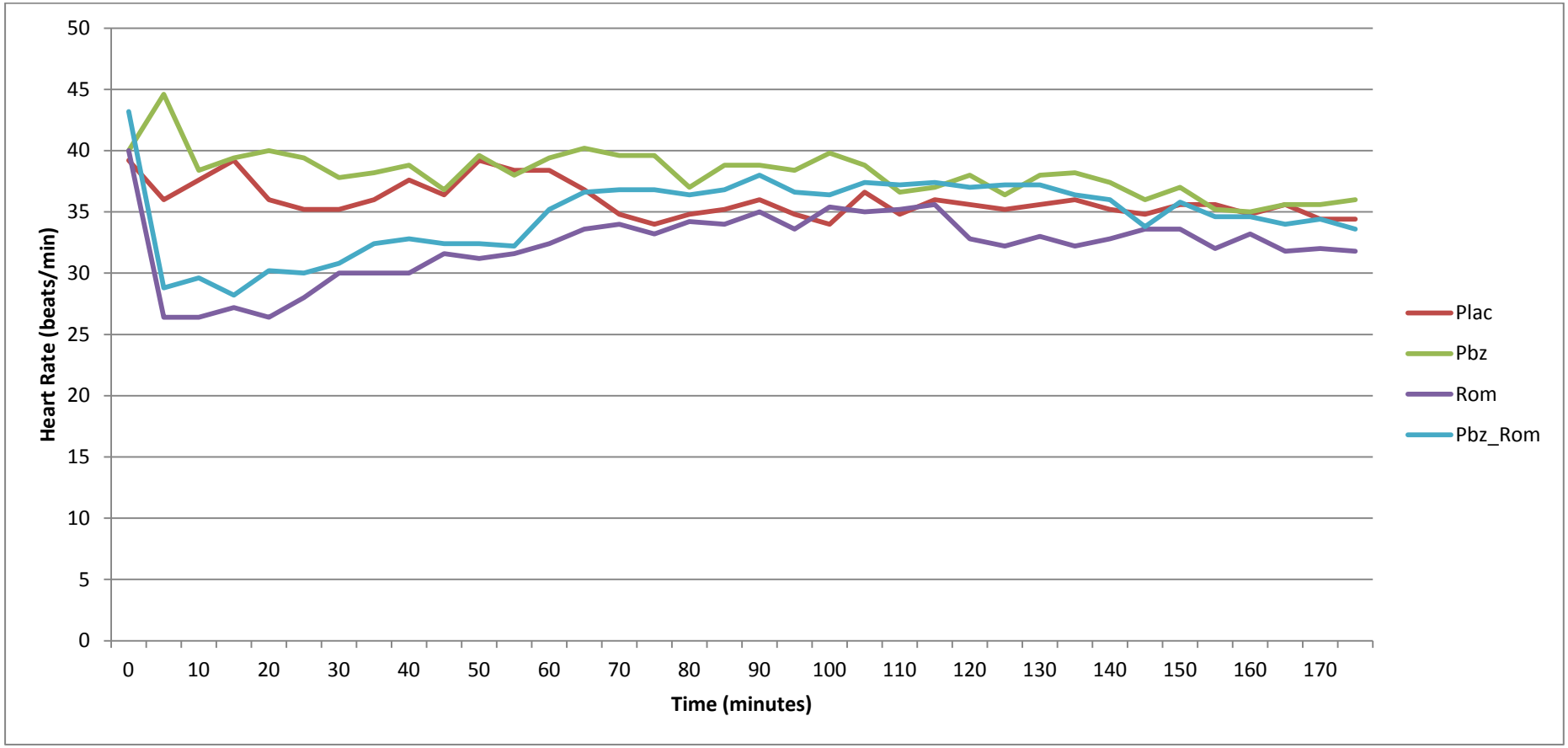

Figure 1.1: Heart rate of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

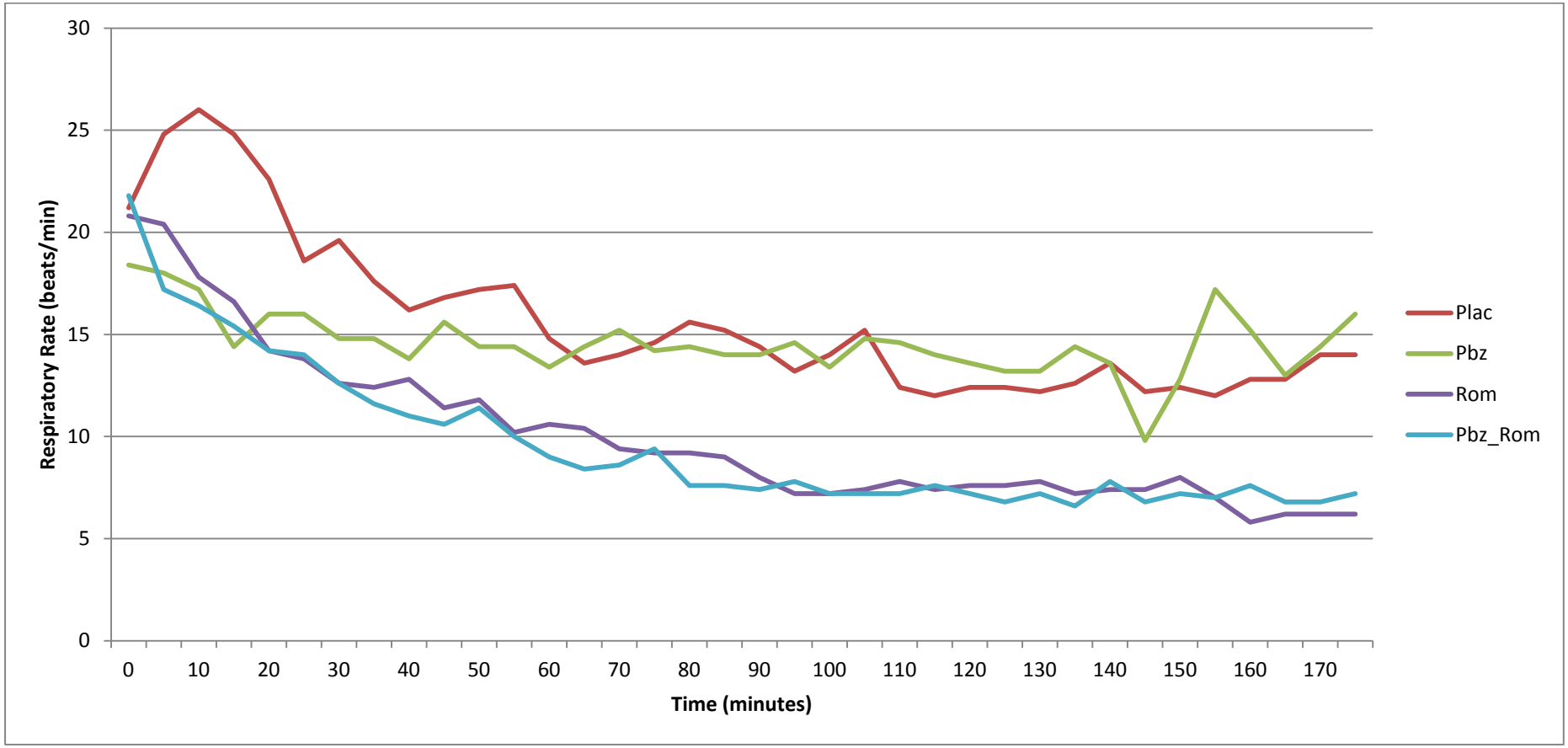

Figure 1.2: Respiratory rate of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

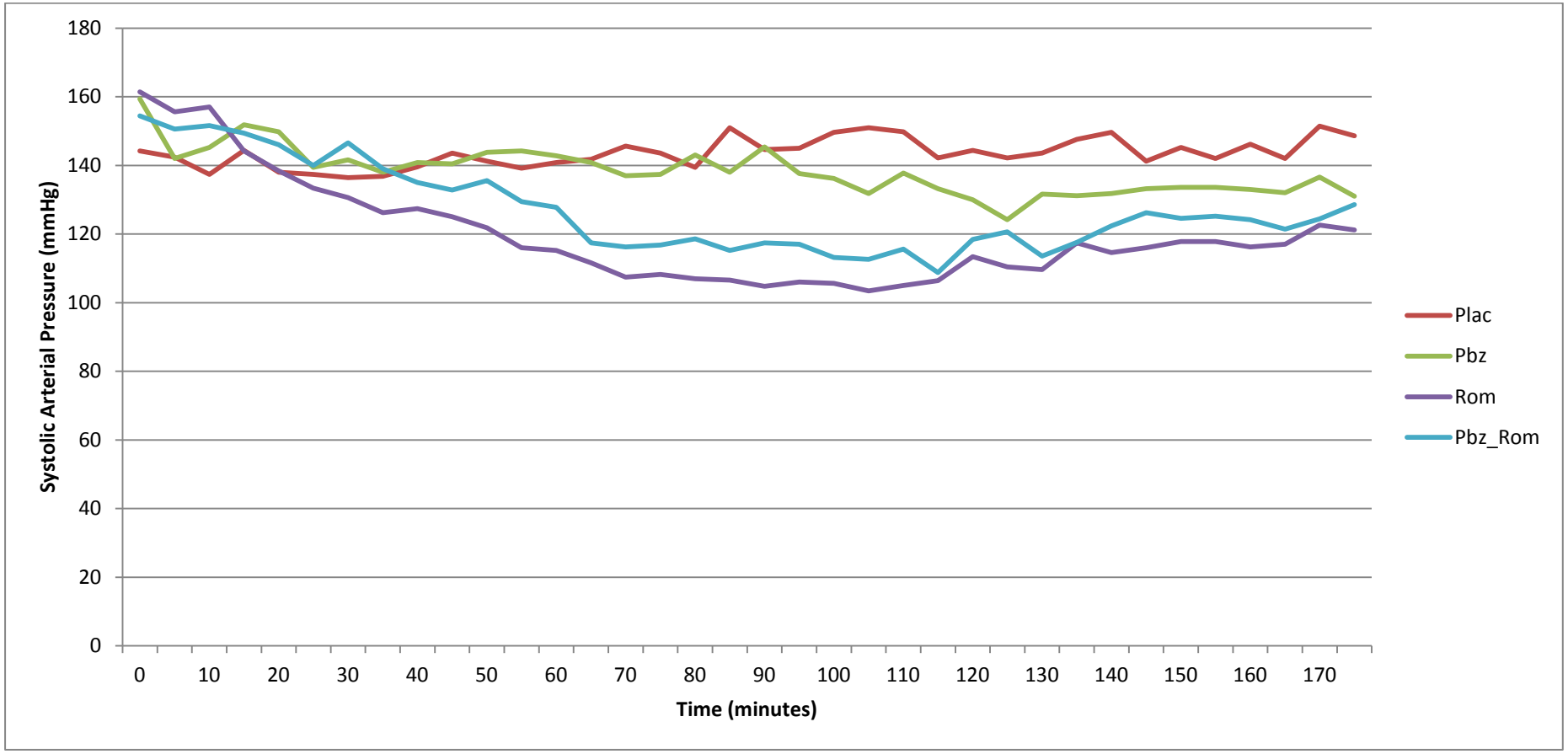

Figure 1.3: Systolic arterial pressure of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

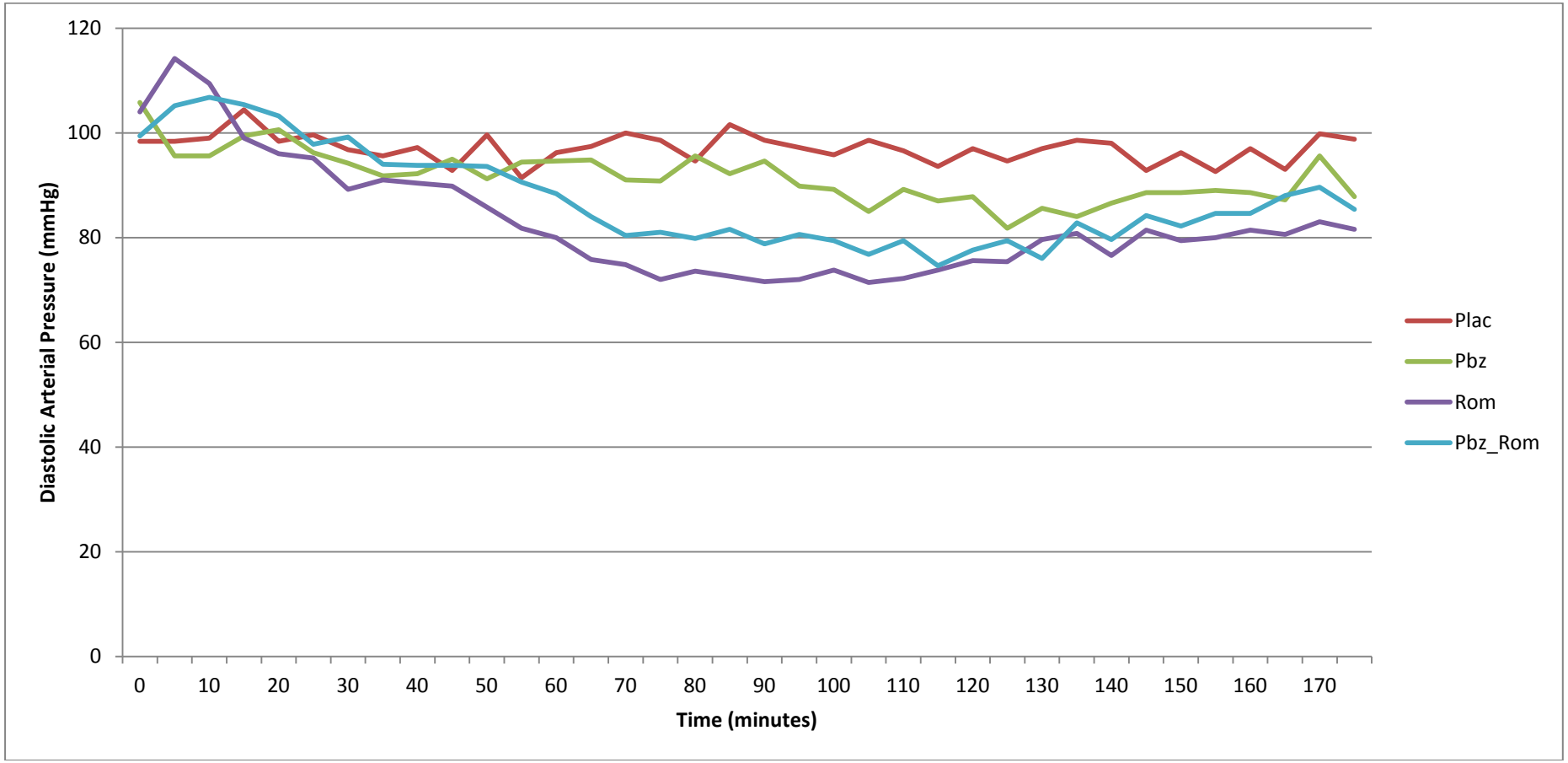

Figure 1.4: Diastolic arterial pressure of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

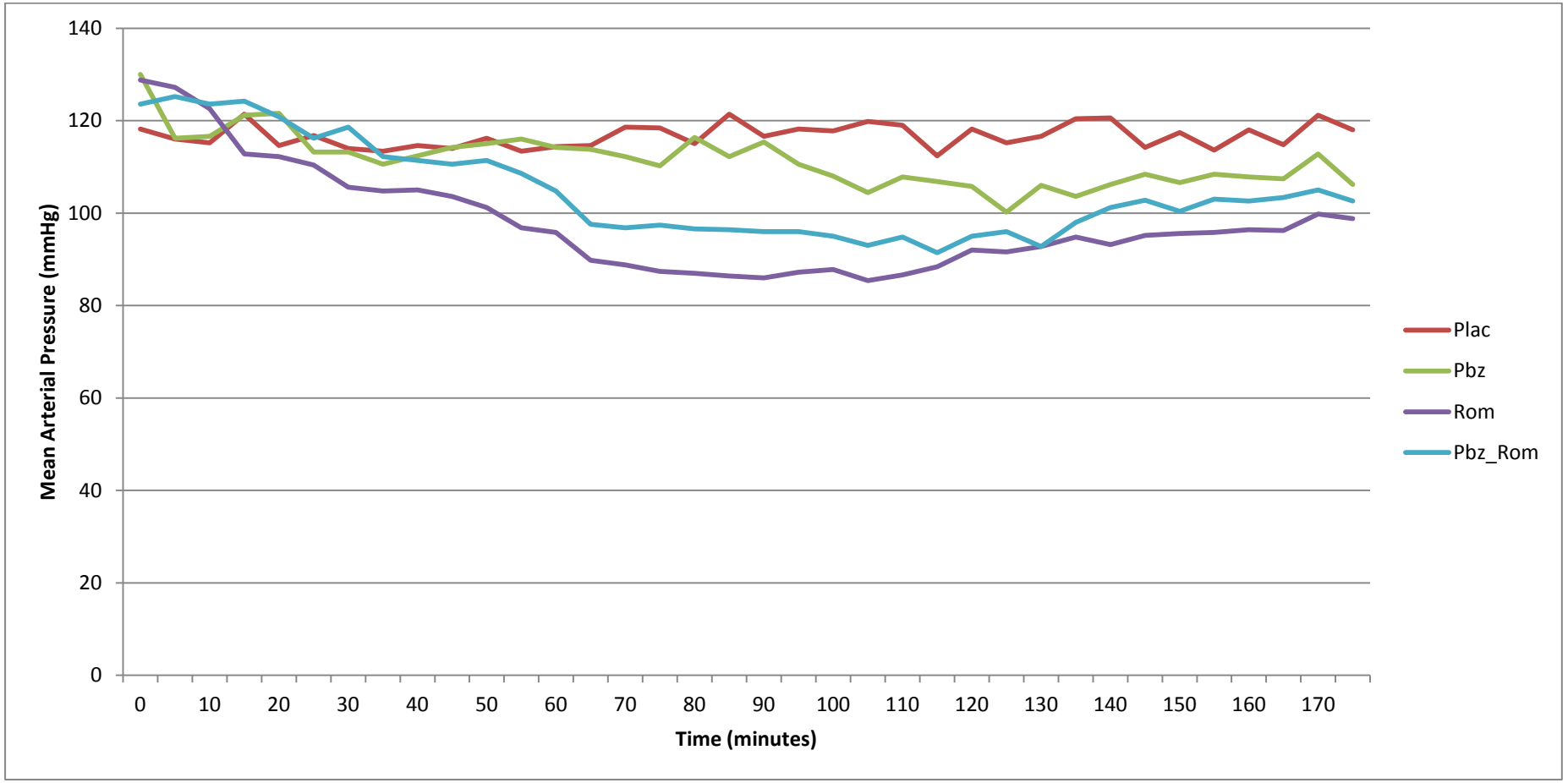

Figure 1.5: Mean arterial pressure of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

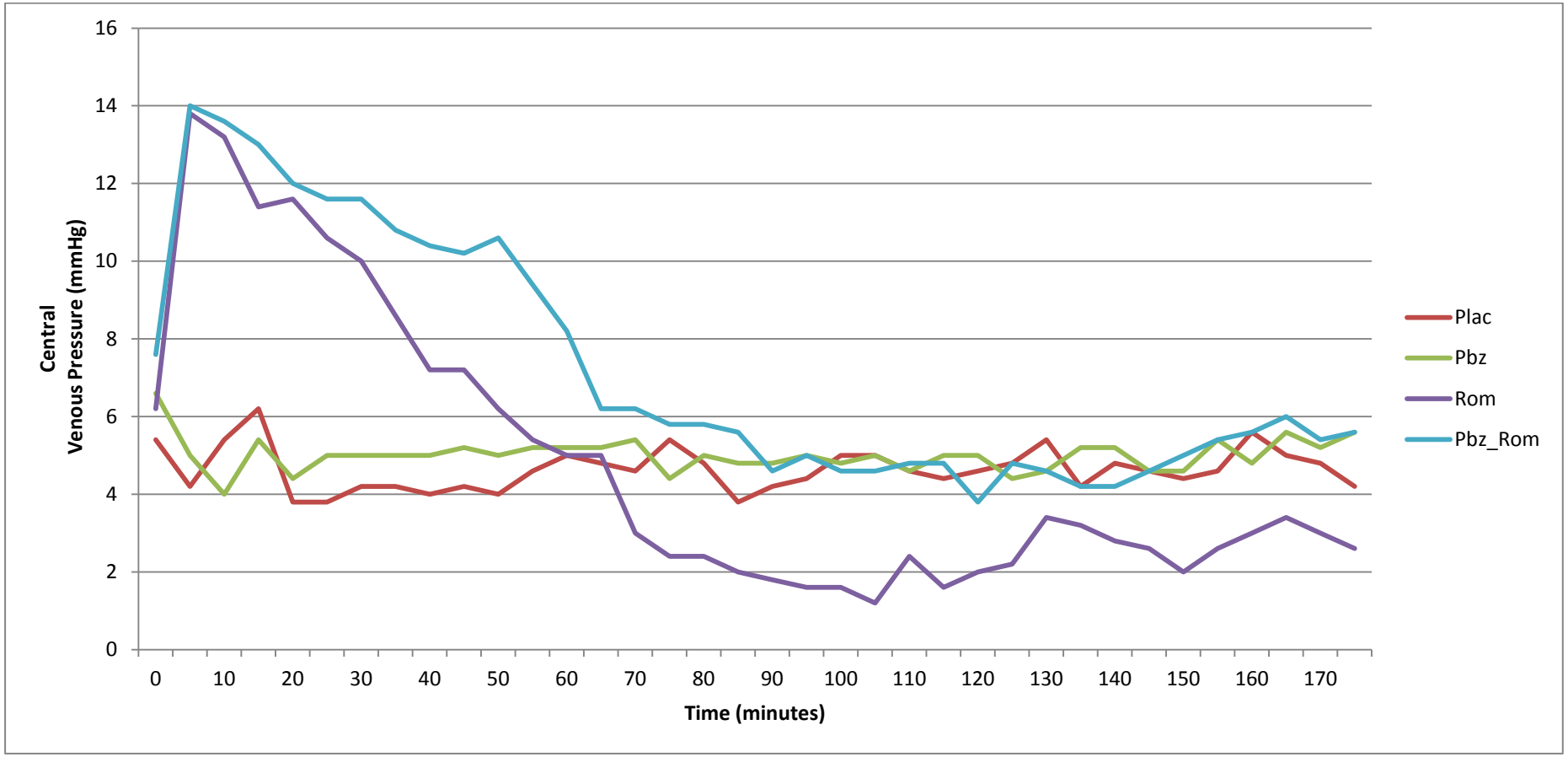

Figure 1.6: Central venous pressure of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

Within-treatment differences / Drug effects over time:

Due to the small amount of horses in the study, repeated measures analysis of variance (the appropriate statistical test) could not be performed. It is therefore not possible to differentiate individual, temporal and physiological variation from drug-induced changes and should be interpreted with caution especially in the light of the fact that most of the parameters remained within normal limits. Changes over time that were observed in the study population are described below.

Heart Rate and Rhythm (Table 3, Fig 1.1)

Two of the five horses in the Plac group and three of the five horses in the Pbz group had intermittent 2^{o} AV blocks. One horse in the Rom group developed SA and 2^{o}AV blocks, and the other four only 2^{o}AV blocks. One horse in the Pbz_Rom group (the same horse that developed SA blocks in the Rom group) developed SA and 2^{0}AV blocks, and the other four only 2^{o}AV blocks.

In the Rom and Pbz_Rom groups, heart rate showed a sharp decline during the first 5 minutes, followed by a gradual increase. The lowest average heart rate was in the Rom group.

There was a small increase in heart rate in the Pbz group at 5 min.

Respiratory Rate (Table 4, Fig 1.2)

Respiratory rate tended to decrease as the 3-hour monitoring period progressed in both the Rom and Pbz_Rom groups.

In the Plac group, respiratory rate increased over the first 10 minutes, however this group did show a larger standard deviation during that time than the other groups.

Arterial Blood Pressure (Tables 5-7, Fig 1.3-1.5)

Systolic arterial pressures showed a decreasing trend for the first 105 minutes in the Rom group and 115 minutes in the Pbz_Rom group, followed by gradual increases. Systolic arterial pressure also tended to decrease slightly in the Pbz group, to a minimum at 125 minutes. The largest decrease, as well as lowest average SAP was seen in the Rom group.

Diastolic arterial pressure tended to increase slightly over the first 5 minutes for Rom and over the first 10 minutes for Pbz_Rom, followed by a gradual decrease to a minimum at 105 minutes for Rom and 115 minutes for Pbz_Rom. Diastolic arterial pressure tended to decrease slightly in the Pbz group, to a minimum at 125 minutes. The largest decrease, as well as lowest average DAP was seen in the Rom group.

Mean arterial pressure decreased to a minimum at 105 minutes in the Rom group and 115 minutes in the Pbz_Rom group respectively, followed by a gradual increase. In the Pbz group, there was a small, gradual trend towards a decreased mean arterial pressure over the course of the monitoring period, with a minimum at 125 minutes. The largest decrease, as well as lowest average MAP was seen in the Rom group.

Central Venous Pressure (Table 8, Fig 1.6)

Central venous pressure increased by more than 100% in the first 5 minutes for Rom and Pbz_Rom, followed by a gradual decrease. In both Rom and Pbz_Rom, the CVP decreased to a level below baseline, with lower values observed in the Rom group than the Pbz_Rom group.

Blood-gas Data

The PCO_2, bicarbonate and base excess tended to increase in groups that received romifidine. PvO_2, potassium and calcium tended to decrease in goups that received romifidine. Data is reported in tables 9.1-16.2 and figures 3.1-3.16.

Sedation

All horses received the same dose (80 µg/kg) of romifidine based on their weight that morning. One horse became heavily sedated, collapsed against the crush and had to be supported to prevent him from falling for both his Pbz_Rom and Rom treatments. Two horses became deeply sedated and significantly ataxic for both Pbz_Rom and Rom treatments. One horse became deeply sedated, but only mildly ataxic for both Pbz_Rom and Rom treatments. One horse became only moderately sedated and mildly ataxic for both Pbz_Rom and Rom treatments. No horses in the Plac or Pbz groups demonstrated any of the measured sedative effects. Sedative effects lasted 45 to 120 min.

Urination

On eight of the ten occasions that horses received romifidine either alone or in combination with phenylbutazone, they urinated between one and four times in the 3-hour monitoring period. Horses that were not treated with romifidine did not urinate during the 3-hour monitoring period.

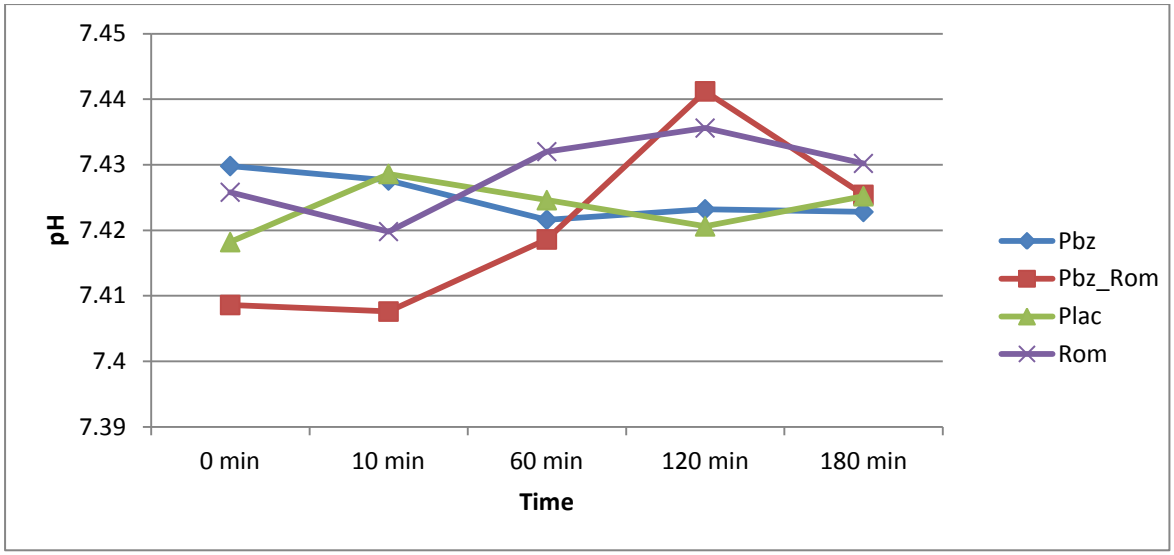

Figure 2.1: Arterial pH of 5 horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

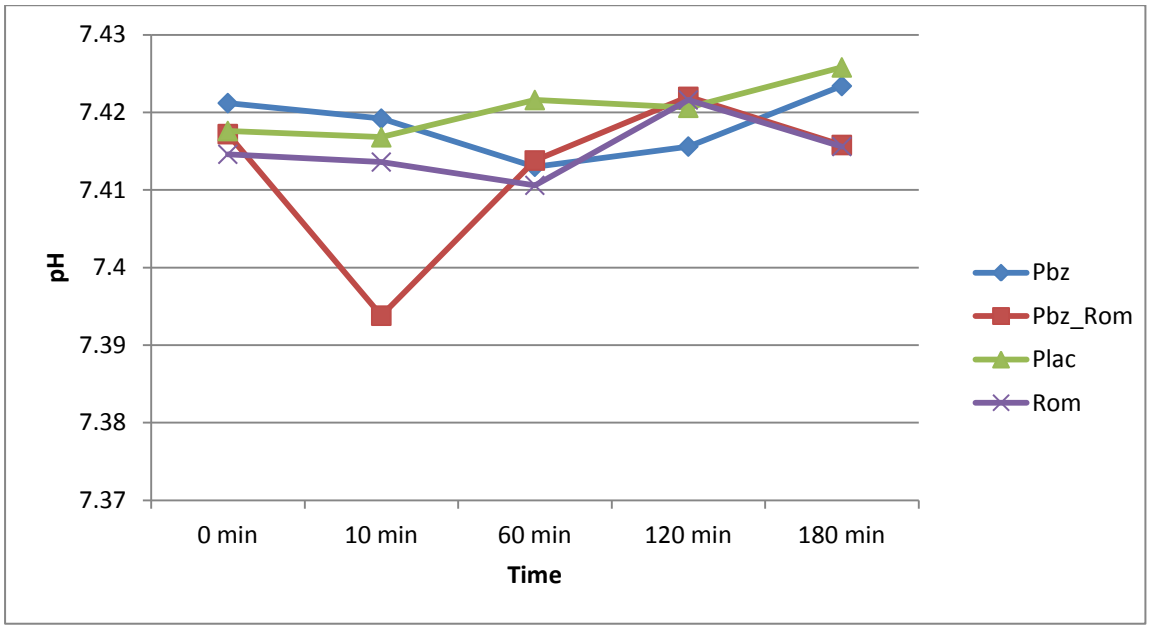

Figure 2.2: Venous pH of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

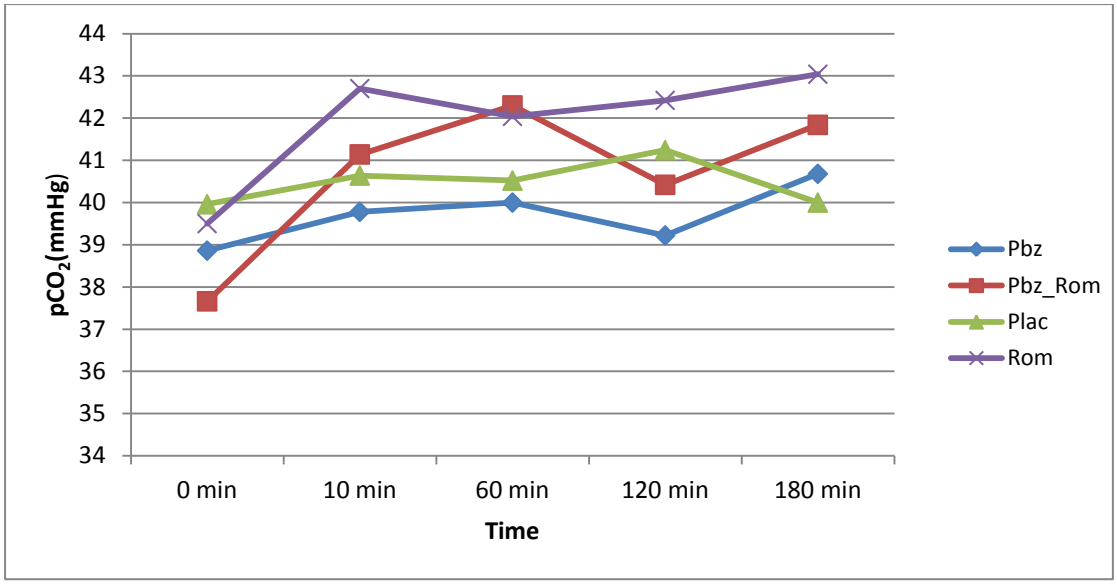

Figure 2.3: $PaCO_2$ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

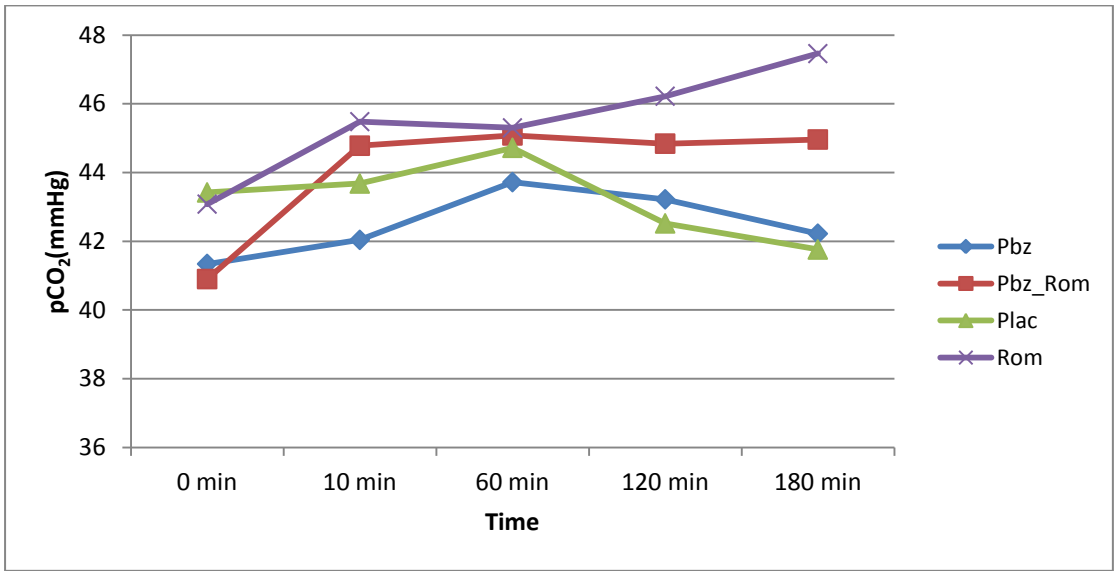

Figure 2.4: $PvCO_2$ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

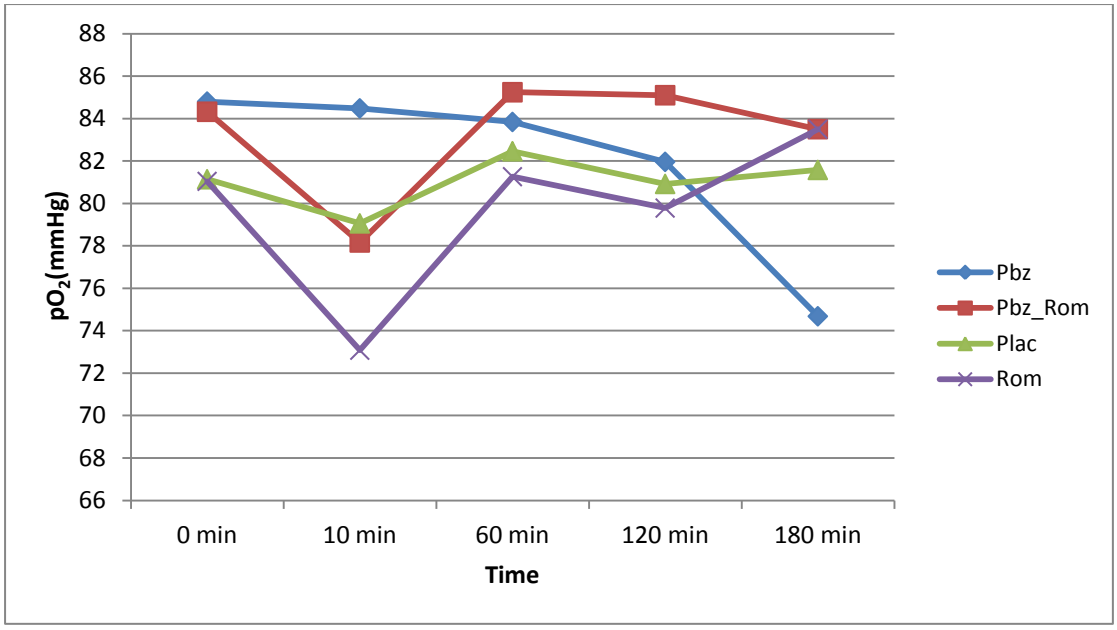

Figure 2.5: PaO_2 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

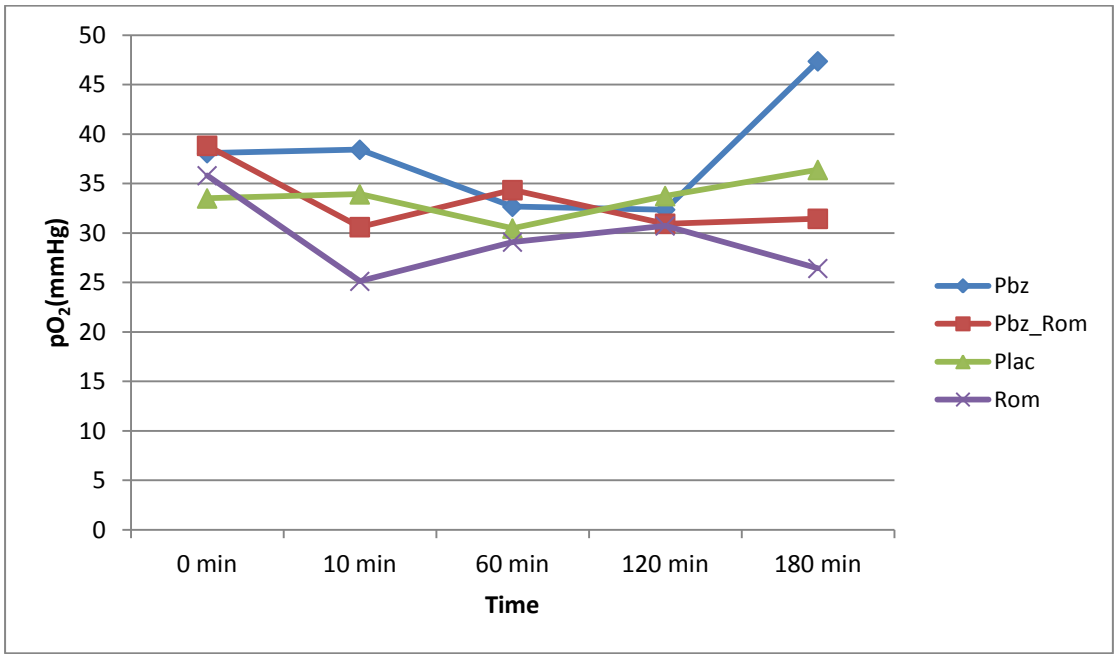

Figure 2.6: PvO_2 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

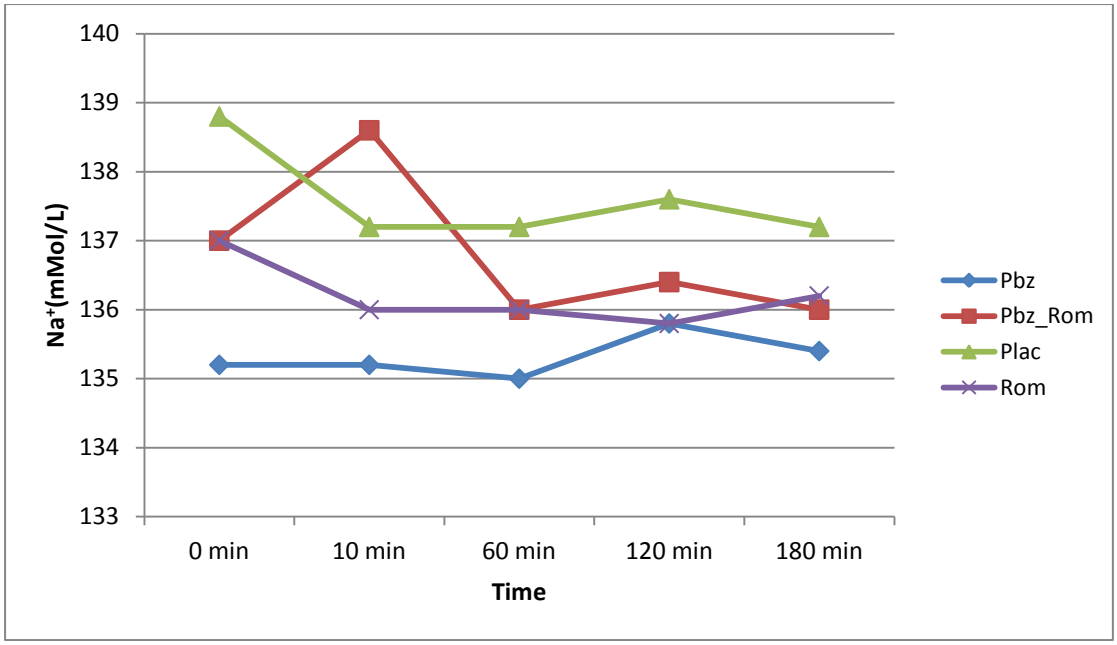

Figure 2.7: Arterial Na^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

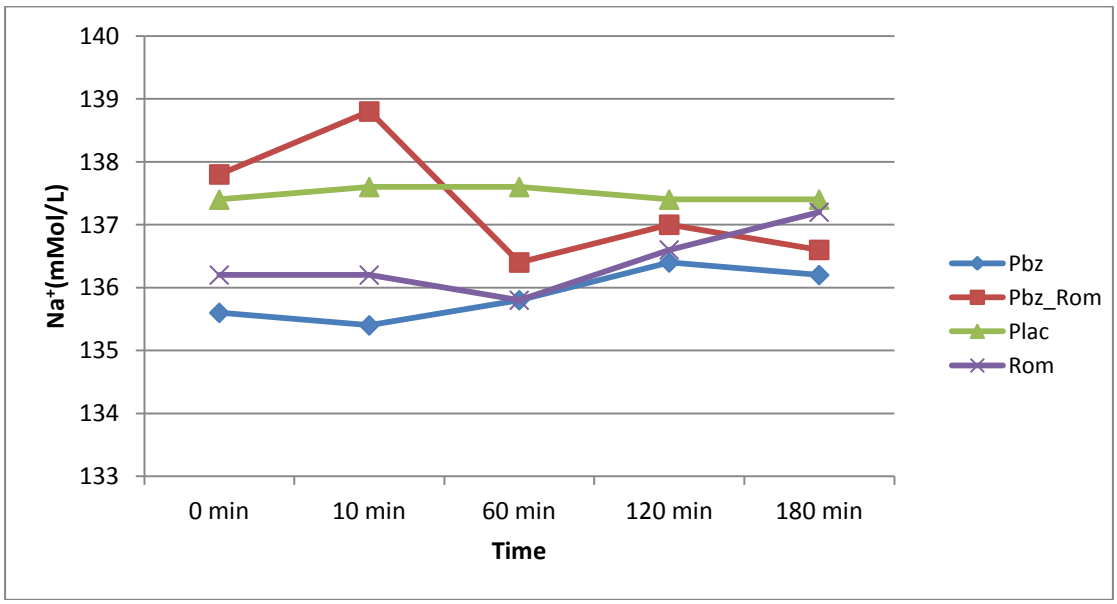

Figure 2.8: Venous Na^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

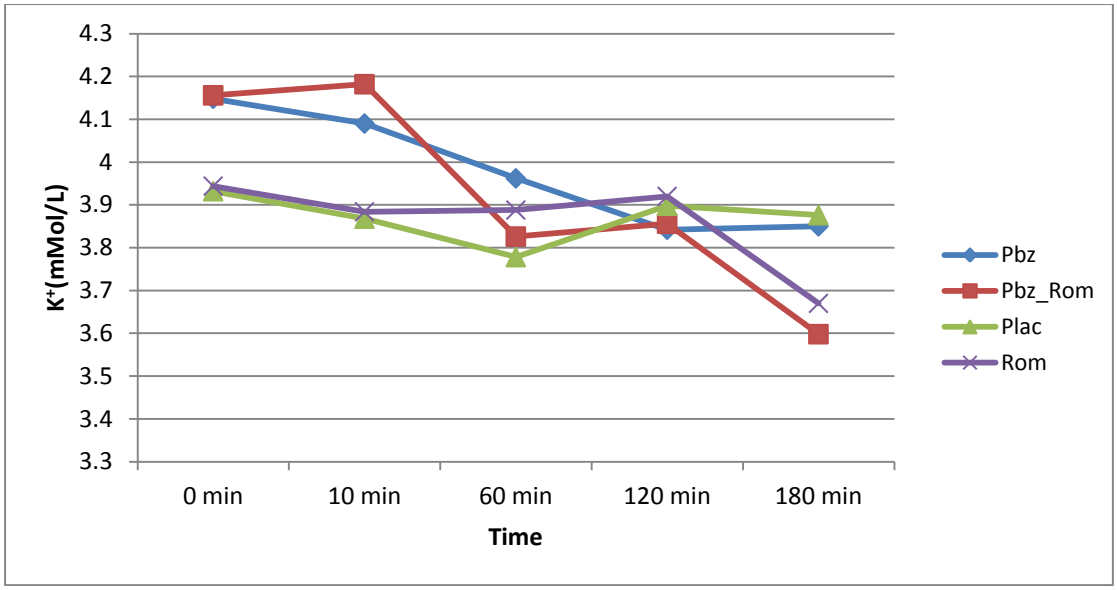

Figure 2.9: Arterial K^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

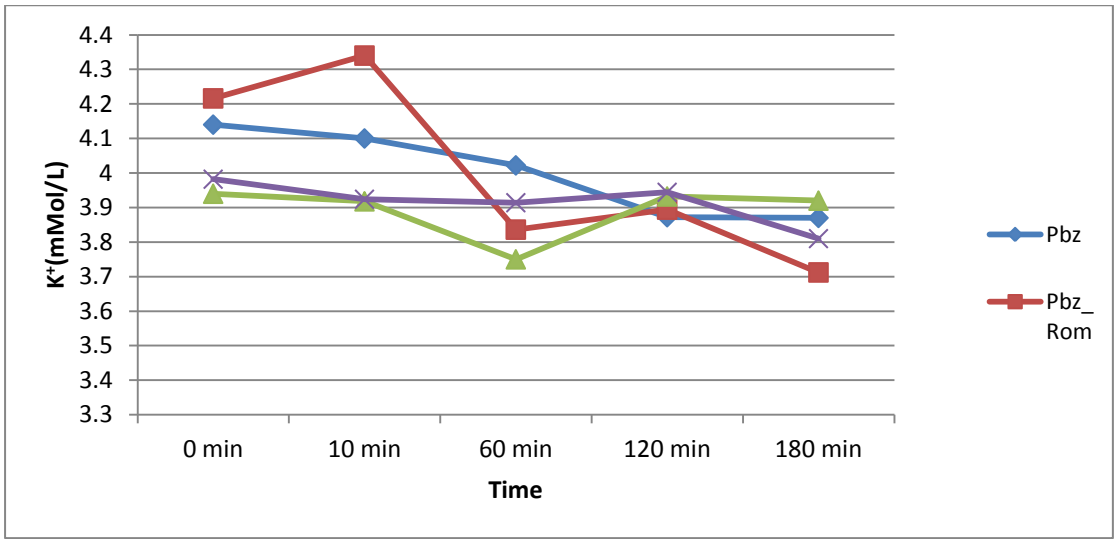

Figure 2.10: Venous K^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

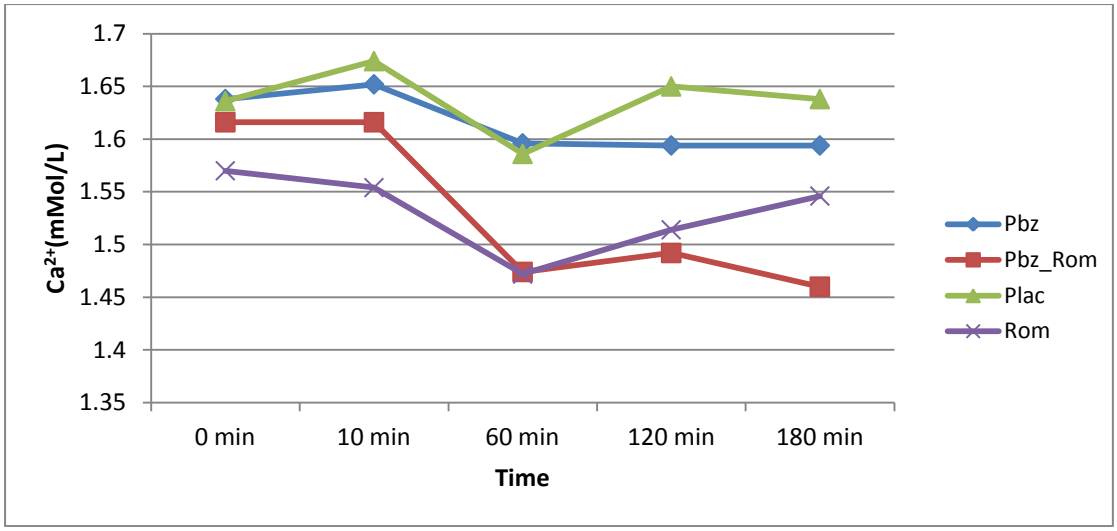

Figure 2.11: Arterial Ca^{2+} of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

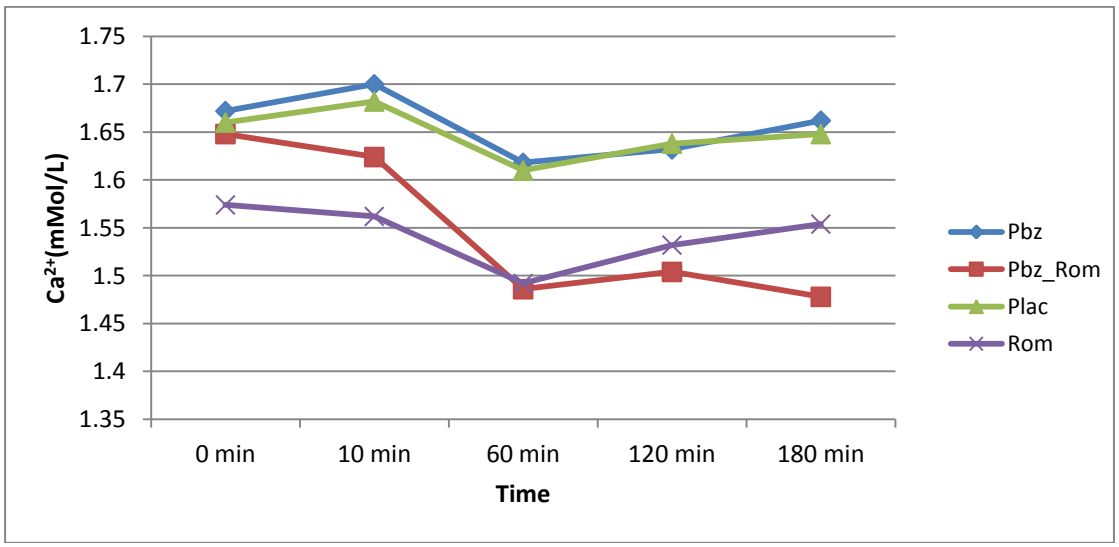

Figure 2.12: Venous Ca^{2+} of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

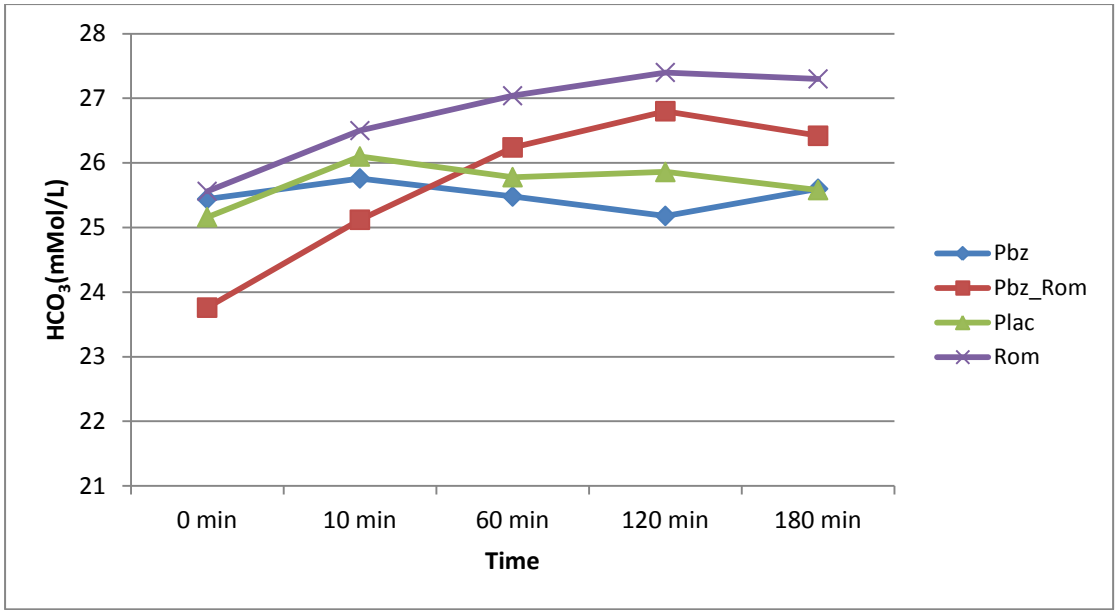

Figure 2.13: Arterial HCO_3 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

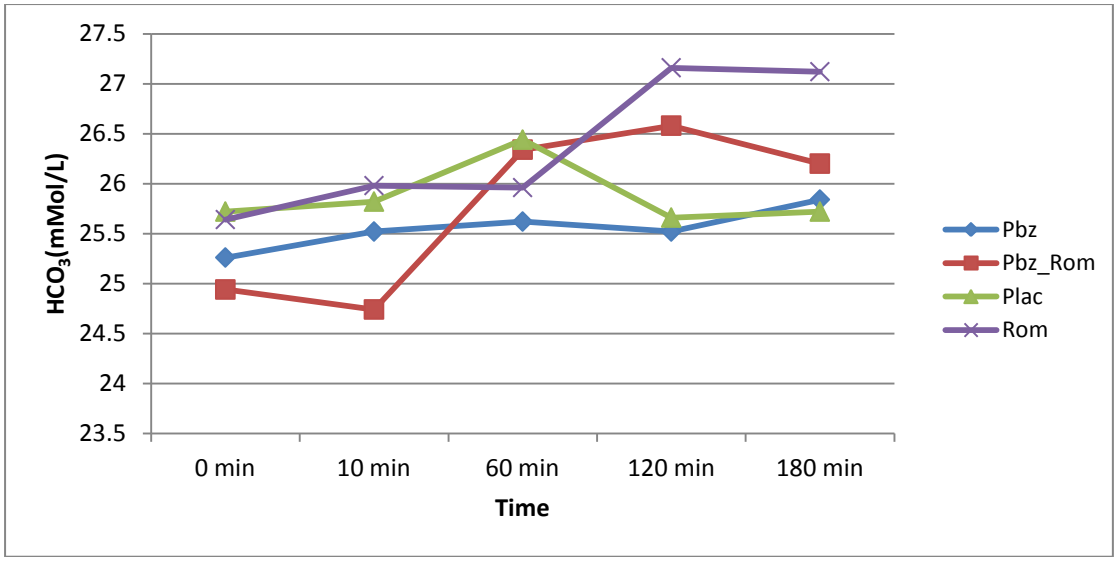

Figure 2.14: Venous HCO_3 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

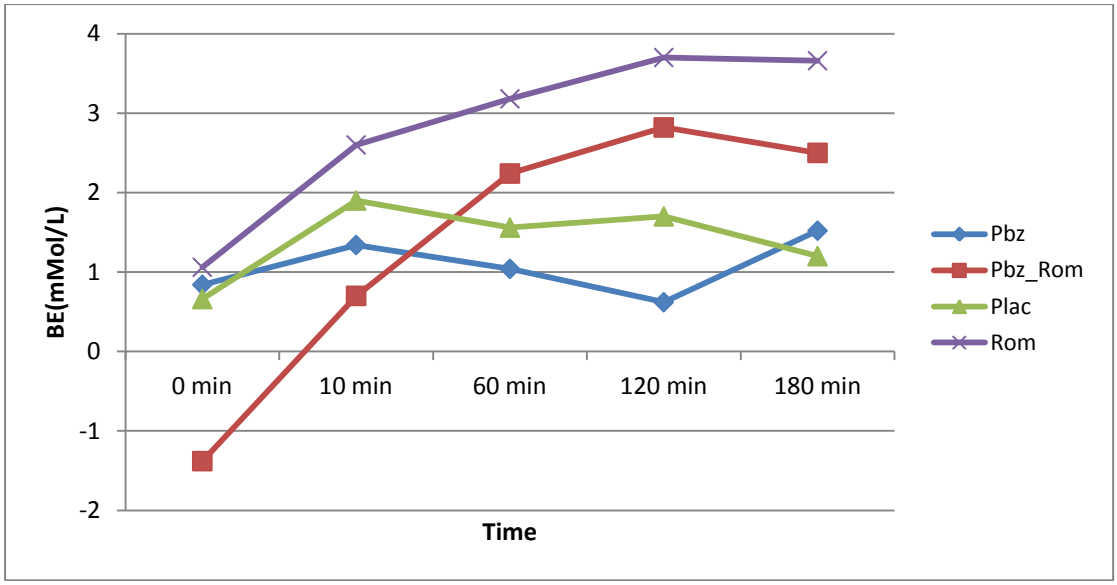

Figure 2.15: Arterial Base Excess (BE) of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

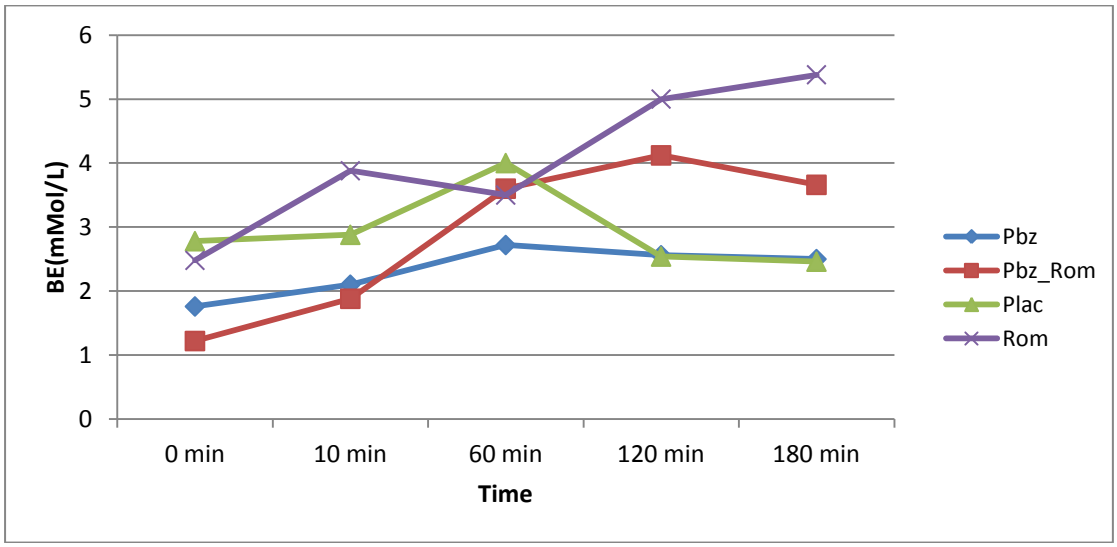

Figure 2.16: Venous Base Excess (BE) of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom).

3.4 Discussion

CLINICAL DATA

Heart Rate and Rhythm

Normal, resting equine heart rate is 28-44 beats/min (Reed, Bayly & Sellon 2003) and appears to be predominantly under parasympathetic control (Hamlin et al. 1972). We expected to find a decrease in heart rate with heart block in both the Rom and Pbz_Rom groups as an acute (<1 min after injection) sequelae to α_2 adrenergic agonist administration (England, Clarke 1996, Browning, Collins 1994, Buhl et al. 2007, Clarke, England & Goossens 1991, Freeman et al. 2002, England, Clarke & Goossens 1992). A low dose (0.005mg/kg) of atropine sulphate administered 5 min prior to romifidine has been shown to counteract the bradycardia produced by romifidine (Gasthuys et al. 1990) and may be helpful in cases where bradycardia with heart block would be extremely undesirable.

The changes in heart rate may be secondary to blood pressure changes, or primary due to romifidine's effect on central and peripheral pre- and post-synaptic α_2- and α_1-adrenergic receptor subtypes. The initial decrease in heart rate and associated heart block is likely due to vagal enhancement of baroreceptor responses to the initial hypertension caused by romifidine (Adams 2001, Murrell, Hellebrekers 2005, Freeman et al. 2002). The subsequent decrease in mean arterial pressure, mediated by α_{2A} and imidazoline receptors in the brainstem (Goodman et al. 2006, Reis, Piletz 1997, Bruban et al. 2002, Murrell, Hellebrekers 2005), may cause a baroreflex-mediated increase in heart rate (Slinker et al. 1982). The overall effect of romifidine on heart rate appears to be mild, yet prolonged, because heart rate is still slightly

decreased at the end of the 3-hour monitoring period, but well within the referance range for horses.

In the data collected during this study, romifidine's effect on heart rate appears to have been mildly attenuated by concurrent phenylbutazone administration. The mild increase in heart rate seen in the Pbz group at 5 min is consistent with previous findings (Brouwer 1987).

Romifidine administration (both alone and in combination with phenylbutazone) caused an increase in the incidence of SA and AV blocks. This effect may result from vagal enhancement of baroreceptor reflex responses to increased blood pressure (Murrell, Hellebrekers 2005). These blocks are not usually clinically significant in horses and do occur under normal circumstances as a consequence of high vagal tone (Reed, Bayly & Sellon 2003). Of greater concern would be the increase in myocardial refractory period caused by this 'artificial' vagal enhancement (Murrell, Hellebrekers 2005). Due to the large size of equine hearts, they are at greater risk of developing sustained re-entrant atrial arrhythmias (Reed, Bayly & Sellon 2003). Theorhetically, α_2 adrenergic agonist administration could increase the risk of these arrhythmias becoming established, as has been the case in other species (Murrell, Hellebrekers 2005). This has however not been reported in horses.

Respiratory Rate

Normal equine respiratory rate is 8-15 breaths/min (Reed, Bayly & Sellon 2003). Both groups that received romifidine had decreases in respiratory rates from pre-sedation values, dropping to as low as 4 breaths / min in individual cases. This respiratory rate decrease is prolonged and respiratory rate is still decreased at the end of the trial observation period. This effect is likely the result of romifidine's effect on pre-synaptic α_{2A} receptors in the brainstem and central imidazoline receptors, which result in decreased central nervous system sympathetic outflow (Goodman et al. 2006, Reis, Piletz 1997, Bruban et al. 2002). Hypoventilation is evident in the blood-gas data (see below) and may cause clinically significant deleterious effects in critically ill patients, patients under general anaesthesia and patients with predisposing respiratory disease.

Arterial Blood Pressure

Normal direct systolic, diastolic and mean arterial pressures in healthy, standing horses are 126-168 mmHg, 85-116 mmHg and 110-133 mmHg respectively (Magdesian 2004). The blood pressure changes in groups that received Romifidine are characterised by an initial hypertension, followed by prolonged hypotension. This is consistent with previous findings for α_2 adrenergic agonists (Goodman et al. 2006, England, Clarke 1996, Murrell, Hellebrekers 2005, Adams 2001, Freeman et al. 2002, Freeman et al. 2000). Maximum change in the first 30 min was not significantly different between treatments for arterial pressure. In the five horses used in this study, arterial pressures decreased most severely in the Rom group, followed by the Pbz_Rom group and with only a very small decrease in the Pbz group. It is interesting to note that administration of phenylbutazone by itself tended to decrease arterial

pressures, but administration of phenylbutazone together with romifidine appeared to attenuate romifidine's depressant effect on arterial blood pressure, however due to the small sample size and lack of statistically significant difference between groups, these observations may be incidental.

Previous authors (Hinchcliff, McKeever & Muir 1994) did not detect a significant decrease in systolic, diastolic and mean arterial pressure after phenylbutazone administration. The trend observed in this study could be incidental or false, since statistical significance could not be determined. If the decrease in arterial pressures is true, different drug formulations and differences in timing of drug administration and monitoring may be causative factors for the discrepancy.

PGE_2 is one of the known ligands for inhibitory heteroreceptors in the adrenergic nervous system, where it inhibits sympathetic neurotransmitter release (Goodman et al. 2006). Inhibiting PGE_2 production may therefore increase sympathetic tone, which would explain the attenuating effect of phenylbutazone on the hypotensive effects of romifidine. Molecular-level research in the future will hopefully shed more light on this subject.

Romifidine causes a gradual decrease in systolic and mean arterial pressures, followed by a gradual recovery. Both systolic and mean arterial pressures are however still decreased below presedation values at 175 min. The sharp initial increase in diastolic pressure is most likely the result of peripheral vasoconstriction, which is mediated by peripheral post-synaptic α_{2B} receptors and possibly also α_1 receptors to a limited extent (Goodman et al. 2006, Adams 2001). The subsequent sharp and then a more

gradual decrease in diastolic pressure, followed by a slow gradual increase indicates a prolonged inhibition on sympathetic outflow and is likely due to the central inhibitory effect of α_{2A} and imidazoline receptor activation (Goodman et al. 2006, Reis, Piletz 1997, Bruban et al. 2002). Diastolic pressure is also still depressed below pre-sedation levels at 175 min. For both groups that received romifidine, arterial blood pressure decreased below established referance ranges. Prolonged hypotension is extremely undesirable, especially in critically ill and anaesthetised patients. Romifidine is frequently used as an adjunct to general anaesthesia, despite the fact that it has been found to increase the risk of anaesthetic death (Johnston et al. 2002). The very long hypotensive effect found in this study may contribute to this increased risk. Atipamezole might be helpful in reversing this prolonged hypotension (Adams 2001, Di Concetto et al. 2007). Unfortunately, when atipamezole is used subsequent to xylazine sedation, only its sedative effect (and not blood pressure suppression) is reversed (Luna, Beale & Taylor 1992). Further studies are required to establish whether the same is true for romifidine.

Central Venous Pressure

Central venous pressure is the intraluminal pressure within the intrathoracic cranial vena cava. It is an indicator of vascular tone, blood volume and cardiac function (Freeman et al. 2002, Magdesian 2004). Echocardiography, clinical pathology and physical examinations ruled out hypovolaemia and cardiac dysfunction as possible causes for abnormalities in CVP in our research population.

The significant difference between Pbz, Rom and Pbz_Rom and Plac found in the maximum change from baseline during the first 30 minutes would suggest that all 3

these treatments had a significant effect on this parameter. The fact that Rom and Pbz_Rom were not significantly different would suggest that there is no exacerbation of effect when these drugs are administered concurrently.

The sharp initial increase in CVP, to a maximum at 5 min, in both the groups that received romifidine is due to vasoconstriction of the peripheral venous vascular bed. The venous system serves as a huge blood reservoir, housing in excess of 60% of circulatory blood (Guyton 1991). Increasing the tone in these vessels can cause a large increase in central venous pressure. After the initial vasoconstiction phase, romifidine causes decreased sympathetic vascular tone, leading to the decreased CVP. The minimum CVP is reached at 105 min (Rom) and 120 min (Pbz_Rom) respectively, whereafter a gradual increase is seen. Romifidine's hypotensive phase appears to outlast this study's 3 hour monitoring period, because blood pressure parameters are still decreased from pre-sedation values at 175 min. The average minimum value for the Rom group drops below the values for Plac and Pbz groups, but the average minimum for the Pbz_Rom group is close to that of the Plac and Pbz groups, which may again indicate an attenuation of romifidine's effect by phenylbutazone.

BLOOD GAS

pH

Normal equine arterial pH is reported to be 7.364-7.484 and venous pH 7.330-7.410 (Robinson 2003). The small differences observed over the trial period are unlikely to be clinically significant, because all values are still within these normal limits. Small increases and decreases in pH may be explained by the decreases and increases in bicarbonate at the same time points.

PCO_2

Normal equine arterial pCO_2 is 34-50 mmHg and venous pCO_2 is 46-64 mmHg (Robinson 2003). All groups that received romifidine showed an increase in arterial and venous pCO_2 over the three hour monitoring period, but values remained within normal limits. This increase results from decreased ventilation due central respiratory suppression. The venous pCO_2 values found in this study are lower than that reported in textbooks, due to the increased altitude. Normal arterial pCO_2 at an altitude of 1300m had been reported to be 37.2 (SD 3.2) mmHg in five percheron mares (Littlejohn, Van Heerden 1975). This value is only marginally lower than the arterial pCO_2 found in the placebo group of this study.

PO_2

Normal equine arterial pO_2 is reported to be 89-115 mmHg (Robinson 2003). The arterial pO_2 found in this study is lower than these values. This can be attributed to the relatively high altitude at the University of Pretoria, which is approximately 1300m above sea level. As altitude increases, arterial oxygen partial pressure decreases. Normal arterial pO_2 at this altitude has been reported for five percheron mares as 75.5 (SD 3.5) mmHg (Littlejohn, Van Heerden 1975), which is lower than the arterial pO_2 found in our placebo group. The reason for this discrepancy is uncertain, but may be related to differences in analyzer sensitivities. This also highlights the importance of establishing a reference range for each individual analyzer.

Arterial oxygenation of animals that received romifidine did not decrease, but venous oxygenation did. Venous oxygenation is often used as a measure of tissue perfusion.

This decrease in venous oxygen tension is in agreement with the findings of previous authors (Freeman et al. 2002). Decreased tissue perfusion is cause for concern in critically ill and anaesthetised patients (Magdesian 2004).

ELECTROLYTES

Changes in electrolytes were too small to be clinically significant, especially in the light of the fact that all electrolyte parameters remained within normal limits except for ionized calcium, which decreased in both groups that received romifidine. The reason for this is uncertain, but it may be related to the α_2 adrenergic agonist's inhibition of vasopressin release (Toribio 2007). Calcium values remained within acceptable limits of 1.5-1.79 mmol/L (Robinson 2003) in all but the Pbz_Rom group, where it fell very slightly below this level.

Bicarbonate and Base Excess

The increase in bicarbonate and base excess in groups that received romifidine are similar to the findings of previous authors (Freeman et al. 2002). The increase in CO_2 caused by hypoventilation would lead to a respiratory acidosis that drives the bicarbonate buffer system in the direction of HCO_3^-. Caution is necessary when interpreting the increase in base excess, since an increase is also seen in the placebo group. Romifidine's effect on these parameters is prolonged beyond the three hour monitoring period.

URINE PRODUCTION

α_2 adrenergic agonists such as romifidine and xylazine are known to increase urine production in horses and ponies (Gasthuys et al. 1996, Thurmon et al. 1984, Trim, Hanson 1986, Gasthuys et al. 1993). This has been associated with an increase in potassium and chloride excretion (Trim, Hanson 1986), which could be a contributing factor to the decrease in plasma potassium. Baroreceptor-mediated inhibition of aldosterone release (in the hypertensive phase) and inhibition of vasopressin-mediated water reabsorption in the collecting duct seem to be responsible for increased urine production (Toribio 2007), which would lead to the increased frequency of urination found in this study.

SEDATION

The differences in duration and depth of sedation in individual animals may be due to differences in basal sympathetic tone, or genetic adrenergic receptor subtype differences. Recent advances in pharmacogenomics show that there are genetic variations of adrenergic receptors within human populations, which influence receptor expression, activation and desensitization (Kirstein, Insel 2004). The same may be true for horses.

4. Conclusion

The following conclusions can be drawn from this study:

1. Phenylbutazone has statistically significant cardiorespiratory effects in the standing horse.

This hypothesis can only be accepted for central venous pressure. The maximum change in CVP during the first 30 min after treatment was statistically significantly greater than that in the placebo group. Systolic, diastolic and mean arterial pressures were not significantly different between groups.

2. Romifidine has cardiorespiratory effects that are prolonged.

This hypothesis cannot be proved nor disproved due to the small sample size. The observed trends would however suggest that it is likely. Romifidine's effects were similar to those found by previous authors (Freeman et al. 2002) in the first two hours, except for heart rate, which was decreased for a longer period. This study found that the depression of heart rate, respiratory rate, systolic-, diastolic- and mean arterial pressure and central venous pressure is prolonged. Minimum values had been reached and these parameters were making a slow recovery, despite still being decreased below pre-sedation values at 3 hours. The increased PCO_2, decreased PvO_2, increased HCO_3^- and actual base excess found up to two hours in previous studies also extends beyond 3 hours. Additionally, romifidine was found to cause a decrease in serum Ca^{2+} and K^+ concentrations. The time frame to 'normal' pre-sedation values of all these parameters is yet to be established.

3. There is an interaction between phenylbutazone and romifidine that has cardiovascular or respiratory effects.

This hypothesis cannot be proved nor disproved due to the small sample size. The adverse events occurring in two previously hospitalised patients led to a suspicion that romifidine and phenylbutazone might exacerbate each other's effects. In the five horses studied, no acute interaction between these drugs was found that could possible explain the acute adverse events occurring in the two patients at the OVAH.

This is a pilot study. Larger trials are required validate the observed findings statistically.

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APPENDIX A

Table 1: Mean (SD) and absolute minimum and maximum cardiovascular and respiratory parameters for five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom) in the first 30 minutes after treatment.

Parameter		Plac	Pbz	Rom	Pbz_Rom
Heart Rate	mean	37(7.1)	40(10.5)	29(8.9)	32(8.2)
(beats/min)	min	28	24	20	20
	max	60	61	48	48
Respiratory Rate	mean	23(14.4)	16(3.2)	17(7.2)	16(6.3)
(breaths/min)	min	8	8	6	8
	max	56	24	40	37
Systolic Arterial	mean	140(12.5)	147(16.0)	146(17.1)	148(17.1)
Pressure	min	123	118	110	111
(mmHg)	max	172	186	176	203
Diastolic Arterial	mean	99(11.8)	98(8.1)	101(11.6)	102(15.6)
Pressure	min	79	81	79	70
(mmHg)	max	120	117	120	132
Mean Arterial	mean	116(12.8)	119(10.5)	117(11.8)	122(13.5)
Pressure	min	95	102	96	95
(mmHg)	max	142	145	141	159
Central Venous	mean	5(3.2)[a,b,c]	5(3.4)[a,d]	11(5.2)[a,e]	12(4.3)[a,d,e]
Pressure	min	-4	-1	-3	3
(mmHg)	Max	8	12	18	18

The same superscript letter indicates that the maximum change from baseline over 30 minutes differed significantly between groups.

Table 2: Mean (SD) and absolute minimum and maximum cardiovascular and respiratory parameters for five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom) over 3 hours.

Parameter		Plac	Pbz	Rom	Pbz_Rom
Heart Rate	mean	36(8.1)	38(10.4)	32(7.5)	35(6.5)
(beats per minute)	min	24	24	20	20
	max	60	64	50	48
Respiratory Rate	mean	16(8.4)	15(4.6)	10(5.4)	10(5.4)
(breaths per minute)	min	4	7	4	4
	max	56	40	40	40
Systolic Arterial Pressure	mean	144(11.8)	138(12.8)	120(19.7)	127(19.7)
(mmHg)	min	120	109	92	86
	max	172	186	176	203
Diastolic Arterial Pressure	mean	97(11.3)	92(8.3)	83(14.4	87(14.2)
(mmHg)	min	65	74	60	52
	max	120	117	120	132
Mean Arterial Pressure	mean	117(11.4)	111(9.5)	98(14.6)	105(15.4)
(mmHg)	min	94	91	74	68
	max	142	145	141	159
Central Venous Pressure	mean	5(3.2)	5(2.6)	5(5.5)	7(4.7)
(mmHg)	min	-4	-1	-6	-3
	max	10	12	18	18

Table 3: Heart Rates of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean (SD).

Time	Plac	Pbz	Rom	Pbz_Rom
0	39.2(12.46)	40.0(12.65)	40.0(7.48)	43.2(8.67)
5	36.0(8.49)	44.6(10.24)	26.4(8.29)	28.8(7.16)
10	37.6(6.69)	38.4(8.76)	26.4(7.80)	29.6(6.07)
15	39.2(5.93)	39.4(12.88)	27.2(7.16)	28.2(8.44)
20	36.0(6.32)	40.0(10.20)	26.4(7.80)	30.2(6.18)
25	35.2(4.38)	39.4(11.82)	28.0(9.38)	30.0(6.60)
30	35.2(6.42)	37.8(11.92)	30.0(10.39)	30.8(6.87)
35	36.0(4)	38.2(13.39)	30.0(10.39)	32.4(7.27)
40	37.6(6.69)	38.8(10.91)	30.0(10.39)	32.8(7.16)
45	36.4(5.73)	36.8(9.12)	31.6(8.99)	32.4(8.99)
50	39.2(7.69)	39.6(14.59)	31.2(10.83)	32.4(7.02)
55	38.4(8.76)	38.0(11.66)	31.6(10.81)	32.2(8.61)
60	38.4(9.21)	39.4(12.60)	32.4(9.84)	35.2(7.60)
65	36.8(7.16)	40.2(13.46)	33.6(9.21)	36.6(6.31)
70	34.8(9.12)	39.6(15.13)	34.0(10.77)	36.8(6.57)
75	34.0(7.75)	39.6(15.13)	33.2(9.12)	36.8(6.57)
80	34.8(7.69)	37.0(11.92)	34.2(9.28)	36.4(6.07)
85	35.2(10.35)	38.8(11.63)	34.0(8.72)	36.8(6.57)
90	36.0(10.95)	38.8(10.92)	35.0(8.37)	38.0(7.45)
95	34.8(9.12)	38.4(8.76)	33.6(7.02)	36.6(6.47)
100	34.0(9.80)	39.8(11.67)	35.4(8.23)	36.4(6.39)
105	36.6(10.43)	38.8(10.35)	35.0(7.48)	37.4(7.06)
110	34.8(9.12)	36.6(10.95)	35.2(5.76)	37.2(6.98)
115	36.0(8)	37.0(12.57)	35.6(5.55)	37.4(7.20)
120	35.6(10.62)	38.0(10.39)	32.8(3.03)	37.0(6.93)
125	35.2(10.83)	36.4(9.42)	32.2(2.86)	37.2(6.87)
130	35.6(10.81)	38.0(12.81)	33.0(6.56)	37.2(5.93)
135	36.0(10.20)	38.2(11.41)	32.2(4.92)	36.4(4.98)
140	35.2(11.10)	37.4(13.70)	32.8(5.22)	36.0(2.83)
145	34.8(11.19)	36.0(12)	33.6(6.23)	33.8(2.86)
150	35.6(9.53)	37.0(11.40)	33.6(5.18)	35.8(3.63)
155	35.6(9.84)	35.2(7.69)	32.0(4.69)	34.6(2.61)
160	34.8(10.06)	35.0(9)	33.2(4.66)	34.6(4.56)
165	35.6(8.29)	35.6(8.53)	31.8(5.02)	34.0(3.94)
170	34.4(9.63)	35.6(8.53)	32.0(5.10)	34.4(4.56)
175	34.4(7.27)	36.0(8.94)	31.8(5.02)	33.6(3.65)

Table 4: Respiratory Rates of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean (SD)

Time	Plac	Pbz	Rom	Pbz_Rom
0	21.2(17.30)	18.4(2.19)	20.8(11.10)	21.8(9.60)
5	24.8(15.34)	18.0(2)	20.4(7.27)	17.2(5.93)
10	26.0(19.70)	17.2(4.38)	17.8(7.63)	16.4(7.30)
15	24.8(19.68)	14.4(4.56)	16.6(7.40)	15.4(6.31)
20	22.6(15.49)	16.0(2.83)	14.2(4.92)	14.2(4.27)
25	18.6(8.17)	16.0(0)	13.8(5.59)	14.0(4.90)
30	19.6(8.76)	14.8(3.90)	12.6(4.34)	12.6(3.29)
35	17.6(7.27)	14.8(3.90)	12.4(4.10)	11.6(2.07)
40	16.2(6.42)	13.8(3.49)	12.8(4.60)	11.0(2.24)
45	16.8(5.76)	15.6(4.98)	11.4(4.88)	10.6(2.19)
50	17.2(5.93)	14.4(3.58)	11.8(4.49)	11.4(3.13)
55	17.4(6.54)	14.4(4.34)	10.2(3.03)	10(2)
60	14.8(6.26)	13.4(4.67)	10.6(2.97)	9.0(2)
65	13.6(4.56)	14.4(3.85)	10.4(3.51)	8.4(2.61)
70	14.0(4)	15.2(3.03)	9.4(2.41)	8.6(2.61)
75	14.6(3.44)	14.2(4.38)	9.2(2.28)	9.4(2.30)
80	15.6(3.58)	14.4(4.56)	9.2(3.11)	7.6(2.61)
85	15.2(3.35)	14.0(3.16)	9.0(3.32)	7.6(2.51)
90	14.4(3.58)	14.0(3.46)	8.0(3.24)	7.4(2.61)
95	13.2(6.57)	14.6(2.61)	7.2(3.03)	7.8(4.60)
100	14.0(5.66)	13.4(4.10)	7.2(3.03)	7.2(2.77)
105	15.2(5.02)	14.8(5.59)	7.4(2.79)	7.2(2.77)
110	12.4(4.56)	14.6(3.29)	7.8(3.27)	7.2(1.64)
115	12.0(5.66)	14.0(3.46)	7.4(2.79)	7.6(2.51)
120	12.4(4.56)	13.6(3.85)	7.6(2.70)	7.2(2.77)
125	12.4(4.56)	13.2(3.90)	7.6(2.70)	6.8(3.11)
130	12.2(2.4)	13.2(4.15)	7.8(2.49)	7.2(5.02)
135	12.6(4.67)	14.4(3.85)	7.2(2.77)	6.6(3.13)
140	13.6(6.07)	13.6(4.34)	7.4(2.70)	7.8(4.66)
145	12.2(4.82)	9.8(5.22)	7.4(1.95)	6.8(2.95)
150	12.4(4.56)	12.8(4.38)	8.0(2.74)	7.2(2.77)
155	12.0(4.69)	17.2(12.85)	7.0(2)	7.0(2.92)
160	12.8(4.38)	15.2(7.56)	5.8(2.49)	7.6(4.83)
165	12.8(4.38)	13.0(4.36)	6.2(2.49)	6.8(3.11)
170	14.0(6)	14.4(7.67)	6.2(2.68)	6.8(3.03)
175	14.0(5.29)	16.0(9.06)	6.2(2.39)	7.2(3.03)

Table 5: Systolic Arterial Pressure of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Plac	Pbz	Rom	Pbz_Rom
0	144.2(14.06)	159.4(17.17)	161.4(4.10)	154.4(15.18)
5	142.4(18.08)	142.0(9.19)	155.6(12.74)	150.6(35.06)
10	137.4(12.58)	145.2(16.57)	157.0(17.42)	151.6(11.70)
15	144.4(9.48)	151.8(18.91)	144.2(13.31)	149.4(15.49)
20	138.0(10.91)	149.8(20.46)	138.4(12.56)	146.0(13.23)
25	137.4(11.84)	139.4(10.36)	133.4(15.04)	140.0(11.51)
30	136.4(14.33)	141.6(16.20)	130.6(17.70)	146.6(13.35)
35	136.8(11.54)	138.0(15.54)	126.2(23.71)	139.0(10.98)
40	139.6(15.27)	140.8(17.34)	127.4(19.62)	135.0(10.77)
45	143.6(9.40)	140.4(12.46)	125.0(17.97)	132.8(12.13)
50	141.2(15.21)	143.8(7.73)	121.8(17.96)	135.6(10.64)
55	139.2(14.24)	144.2(6.14)	116.0(13.66)	129.4(15.68)
60	140.8(11.69)	142.8(10.38)	115.2(12.38)	127.8(8.11)
65	141.8(13.66)	140.8(10.57)	111.6(10.76)	117.4(11.72)
70	145.6(11.13)	137.0(8.46)	107.4(12.95)	116.2(13.92)
75	143.6(12.03)	137.4(11.35)	108.2(12.09)	116.8(17.57)
80	139.4(8.65)	143.0(9.30)	107.0(11.94)	118.6(18.31)
85	151.0(16.48)	138.0(9.51)	106.6(13.85)	115.2(24.41)
90	144.6(15.69)	145.4(10.69)	104.8(10.71)	117.4(21.77)
95	145.0(9.82)	137.6(12.26)	106.0(13.36)	117.0(23.63)
100	149.6(13.85)	136.2(9.47)	105.6(10.57)	113.2(90.01)
105	151.0(11.18)	131.8(12.44)	103.4(8.38)	112.6(19.77)
110	149.8(19.25)	137.8(11.76)	105.0(8.34)	115.6(15.53)
115	142.2(12.91)	133.2(8.70)	106.4(8.29)	108.8(13.97)
120	144.4(8.23)	130.0(12.17)	113.4(11.41)	118.4(16.36)
125	142.2(10.89)	124.2(7.56)	110.4(11.46)	120.6(23.54)
130	143.6(10.45)	131.6(11.28)	109.6(9.13)	113.6(17.77)
135	147.6(6.88)	131.2(10.62)	117.4(18.04)	117.6(20.13)
140	149.6(7.92)	131.8(12.91)	114.6(12.93)	122.4(17.36)
145	141.2(7.36)	133.2(12.15)	116.0(18.12)	126.2(13.74)
150	145.2(13.31)	133.6(11.95)	117.8(16.56)	124.6(14.10)
155	142.0(9.35)	133.6(9.53)	117.8(17.73)	125.2(16.95)
160	146.2(8.04)	133.0(9.33)	116.2(13.07)	124.2(15.06)
165	142.0(10.84)	132.0(10.37)	117.0(12.21)	121.4(13.69)
170	151.4(11.63)	136.6(5.81)	122.6(14.79)	124.4(12.26)
175	148.6(10.43)	131.0(10.56)	121.2(14.94)	128.6(14.05)

Table 6: Diastolic Arterial Pressure of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Plac	Pbz	Rom	Pbz_Rom
0	98.4(11.65)	105.8(9.15)	104.0(6.86)	99.4(6.95)
5	98.4(14.69)	95.6(7.86)	114.2(3.77)	105.2(24.16)
10	99.0(14.75)	95.6(8.50)	109.4(9.32)	106.8(15.80)
15	104.4(10.78)	99.4(8.68)	99.0(11.64)	105.4(14.72)
20	98.4(13.85)	100.6(5.59)	96.0(6.48)	103.2(17.06)
25	99.6(9.58)	96.2(8.07)	95.2(10.35)	97.8(14.96)
30	96.8(13.35)	94.2(7.12)	89.2(11.71)	99.2(18.78)
35	95.6(11.72)	91.8(6.61)	91.0(13.64)	94.0(16.31)
40	97.2(12.19)	92.2(6.98)	90.4(14.77)	93.8(11.32)
45	92.8(17.36)	95(4.24)	89.8(12.97)	93.8(10.33)
50	99.6(12.54)	91.2(9.12)	85.8(13.10)	93.6(7.83)
55	91.4(17.73)	94.4(3.78)	81.8(11.12)	90.6(5.18)
60	96.2(11.97)	94.6(5.50)	80.0(12.25)	88.4(4.04)
65	97.4(12.74)	94.8(8.07)	75.8(10.71)	84.0(7.65)
70	100.0(10.56)	91(4.36)	74.8(10.62)	80.4(6.58)
75	98.6(10.41)	90.8(7.89)	72.0(9.27)	81.0(11.81)
80	94.6(10.09)	95.6(5.22)	73.6(9.45)	79.8(12.26)
85	101.6(11.72)	92.2(7.05)	72.6(9.40)	81.6(14.64)
90	98.6(14.91)	94.6(6.91)	71.6(7.37)	78.8(16.75)
95	97.2(9.63)	89.8(7.40)	72.0(9.87)	80.6(14.50)
100	95.8(14.53)	89.2(6.69)	73.8(8.50)	79.4(12.62)
105	98.6(10.01)	85(5.48)	71.4(9.40)	76.8(11.37)
110	96.6(17.47)	89.2(6.06)	72.2(11.28)	79.4(15.69)
115	93.6(13.58)	87(7.78)	73.8(9.78)	74.6(10.64)
120	97.0(7.45)	87.8(7.26)	75.6(8.32)	77.6(10.90)
125	94.6(10.83)	81.8(4.49)	75.4(8.76)	79.4(16.23)
130	97.0(10.37)	85.6(7.96)	79.6(14.67)	76.0(8.86)
135	98.6(8.38)	84(6.63)	80.8(13.39)	82.8(11.01)
140	98.0(10.68)	86.6(7.57)	76.6(10.85)	79.6(8.68)
145	92.8(12.74)	88.6(12.12)	81.4(13.13)	84.2(7.50)
150	96.2(12.19)	88.6(9.40)	79.4(11.87)	82.2(8.67)
155	92.6(10.06)	89(5.79)	80.0(12.77)	84.6(8.50)
160	97.0(9.97)	88.6(8.20)	81.4(7.50)	84.6(6.43)
165	93.0(8.72)	87.2(4.60)	80.6(8.53)	88.0(10.05)
170	99.8(9.83)	95.6(10.85)	83.9(8.46)	89.6(4.39)
175	98.8(10.38)	87.8(10.06)	81.6(7.64)	85.4(3.97)

Table 7: Mean Arterial Pressure of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Plac	Pbz	Rom	Pbz_Rom
0	118.2(13.27)	130(10.98)	128.8(7.79)	123.6(8.50)
5	116.0(17.51)	116.2(6.80)	127.2(6.69)	125.2(25.02)
10	115.2(14.02)	116.6(9.94)	122.6(6.62)	123.6(11.80)
15	121.4(11.13)	121.2(12.24)	112.8(13.03)	124.2(13.31)
20	114.6(15.11)	121.6(11.97)	112.2(7.92)	120.8(13.33)
25	116.8(9.98)	113.2(7.53)	110.4(8.73)	116.2(10.73)
30	114.0(14.75)	113.2(7.69)	105.6(10.36)	118.6(12.88)
35	113.4(12.10)	110.6(7.44)	104.8(13.68)	112.2(10.55)
40	114.6(13.69)	112.4(10.62)	105.0(14.18)	111.4(9.21)
45	114.0(13.19)	114.2(6.22)	103.6(12.28)	110.6(10.71)
50	116.2(15.21)	115.0(4.06)	101.2(12.60)	111.4(8.32)
55	113.4(12.93)	116.0(4.42)	96.8(10.80)	108.6(8.79)
60	114.4(12.58)	114.2(6.18)	95.8(11.37)	104.8(5.54)
65	114.6(12.60)	113.8(6.53)	89.8(9.73)	97.6(7.44)
70	118.6(11.55)	112.2(4.97)	88.8(11.80)	96.8(8.23)
75	118.4(12.01)	110.2(10.06)	87.4(9.42)	97.4(14.17)
80	115.0(11)	116.4(5.41)	87.0(9.43)	96.6(16.20)
85	121.4(12.93)	112.2(7.82)	86.4(7.57)	96.4(18.50)
90	116.6(15.40)	115.4(9.91)	86.0(6.20)	96.0(18.96)
95	118.2(10.11)	110.6(9.61)	87.2(10.80)	96.0(16.91)
100	117.8(13.07)	108.0(7)	87.8(7.56)	95.0(14.88)
105	119.8(9.26)	104.4(7.16)	85.4(7.83)	93.0(13.66)
110	119.0(16.36)	107.8(8.84)	86.6(10.16)	94.8(15.93)
115	112.4(14.81)	106.8(8.73)	88.4(9.10)	91.4(11.65)
120	118.2(13.27)	105.8(8.41)	92.0(8.46)	95.0(14.20)
125	115.2(9.96)	100.2(5.81)	91.6(9.07)	96.0(18.87)
130	116.6(10.99)	106.0(9.14)	92.8(10.23)	92.8(11.76)
135	120.4(7.64)	103.6(9.37)	94.8(12.70)	98.0(15.36)
140	120.6(9.63)	106.2(9.52)	93.2(10.35)	101.2(8.79)
145	114.2(9.68)	108.4(12.76)	95.2(12.97)	102.8(10.47)
150	117.4(13.07)	106.6(9.18)	95.6(11.91)	100.4(11.01)
155	113.6(11.37)	108.4(5.98)	95.8(12.64)	103.0(11.90)
160	118.0(10.51)	107.8(7.19)	96.4(8.53)	102.6(9.94)
165	114.8(11.08)	107.4(5.59)	96.2(7.89)	103.4(8.71)
170	121.2(9.26)	112.8(7.53)	99.8(8.98)	105.0(4.30)
175	118.0(12.98)	106.2(9.31)	98.8(9.55)	102.6(8.59)

Table 8: Central Venous Pressure of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Plac	Pbz	Rom	Pbz_Rom
0	5.4(2.30)	6.6(3.36)	6.2(6.69)	7.6(2.70)
5	4.2(4.21)	5.0(3.54)	13.8(4.92)	14.0(4.80)
10	5.4(1.34)	4.0(3.94)	13.2(4.87)	13.6(4.62)
15	6.2(1.64)	5.4(3.78)	11.4(5.27)	13.0(3.67)
20	3.8(4.60)	4.4(3.58)	11.6(4.51)	12.0(4)
25	3.8(4.44)	5.0(3.54)	10.6(4.28)	11.6(3.97)
30	4.2(3.70)	5.0(3.39)	10.0(4.47)	11.6(5.08)
35	4.2(3.56)	5.0(2.92)	8.6(5.37)	10.8(3.77)
40	4(3.46)	5.0(1.87)	7.2(5.36)	10.4(3.44)
45	4.2(4.21)	5.2(2.59)	7.2(5.02)	10.2(3.19)
50	4(4.06)	5.0(2.92)	6.2(4.49)	10.6(3.44)
55	4.6(4.39)	5.2(2.95)	5.4(4.67)	9.4(3.29)
60	5(4.06)	5.2(2.77)	5.0(5.79)	8.2(2.95)
65	4.8(4.38)	5.2(2.77)	5.0(6.28)	6.2(3.70)
70	4.6(3.78)	5.4(3.21)	3.0(4.47)	6.2(3.96)
75	5.4(4.15)	4.4(3.29)	2.4(4.67)	5.8(3.96)
80	4.8(3.96)	5.0(2.83)	2.4(3.97)	5.8(4.82)
85	3.8(4.15)	4.8(2.68)	2.0(3.32)	5.6(4.56)
90	4.2(3.70)	4.8(3.11)	1.8(4.02)	4.6(4.62)
95	4.4(3.36)	5.0(2.35)	1.6(3.44)	5.0(4.36)
100	5(3.54)	4.8(2.39)	1.6(3.58)	4.6(4.77)
105	5(3.67)	5.0(2.55)	1.2(3.83)	4.6(4.22)
110	4.6(3.51)	4.6(1.67)	2.4(2.97)	4.8(4.71)
115	4.4(2.70)	5.0(2.12)	1.6(2.97)	4.8(4.60)
120	4.6(2.30)	5.0(1.87)	2.0(4.24)	3.8(4.82)
125	4.8(2.28)	4.4(2.07)	2.2(3.70)	4.8(4.15)
130	5.4(2.30)	4.6(2.07)	3.4(4.22)	4.6(3.78)
135	4.2(2.49)	5.2(2.77)	3.2(5.02)	4.2(3.56)
140	4.8(3.49)	5.2(3.11)	2.8(5.02)	4.2(3.27)
145	4.6(3.36)	4.6(3.58)	2.6(4.39)	4.6(3.78)
150	4.4(3.29)	4.6(2.41)	2.0(5.43)	5.0(2.92)
155	4.6(3.21)	5.4(2.30)	2.6(5.03)	5.4(2.97)
160	5.6(4.04)	4.8(2.39)	3.0(4.53)	5.6(3.29)
165	5(3.16)	5.6(2.70)	3.4(4.51)	6.0(2.45)
170	4.8(2.77)	5.2(2.28)	3.0(4.64)	5.4(3.51)
175	4.2(2.28)	5.6(2.51)	2.6(4.22)	5.6(3.13)

Table 9.1: Arterial pH of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD)

Time	Arterial pH			
	Plac	Pbz	Rom	Pbz_Rom
0 min	7.42(0.04)	7.43(0.03)	7.43(0.02)	7.41(0.02)
10 min	7.43(0.04)	7.43(0.03)	7.42(0.03)	7.41(0.03)
60 min	7.42(0.03)	7.42(0.02)	7.43(0.02)	7.42(0.02)
120 min	7.42(0.02)	7.420.02)	7.44(0.03)	7.44(0.02)
180 min	7.43(0.02)	7.42(0.04)	7.43(0.03)	7.43(0.02)

Table 9.2: Venous pH of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous pH			
	Plac	Pbz	Rom	Pbz_Rom
0 min	7.42(0.03)	7.42(0.03)	7.41(0.03)	7.42(0.02)
10 min	7.42(0.03)	7.42(0.03)	7.41(0.03)	7.39(0.02)
60 min	7.42(0.02)	7.41(0.03)	7.41(0.03)	7.41(0.02)
120 min	7.42(0.02)	7.42(0.03)	7.42(0.03)	7.42(0.02)
180 min	7.43(0.02)	7.42(0.02)	7.42(0.03)	7.42(0.02)

Table 10.1: Arterial pCO_2 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial pCO_2			
	Plac	Pbz	Rom	Pbz_Rom
0 min	39.96(3.03)	38.86(2.73)	39.50(1.55)	37.66(3.75)
10 min	40.64(3.11)	39.78(1.69)	42.70(2.25)	41.14(2.88)
60 min	40.52(2.25)	40(1.29)	42.04(2.84)	42.30(2.61)
120 min	41.24(2.57)	39.22(1.60)	42.42(1.58)	40.42(1.20)
180 min	40.00(2.01)	40.68(2.95)	43.04(0.96)	41.84(2.38)

Table 10.2: Venous pCO_2 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous pCO2			
	Plac	Pbz	Rom	Pbz_Rom
0 min	43.42(3.55)	41.34(3.07)	43.08(1.92)	40.90(1.96)
10 min	43.68(3.41)	42.04(1.72)	45.48(0.90)	44.78(0.96)
60 min	44.72(2.18)	43.72(1.85)	45.30(3.94)	45.08(1.26)
120 min	42.52(3.54)	43.22(2.72)	46.22(1.64)	44.84(0.89)
180 min	41.76(0.93)	42.22(3.36)	47.46(2.74)	44.96(2.17)

Table 11.1: Arterial pO_2 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial pO_2			
	Plac	Pbz	Rom	Pbz_Rom
0 min	81.14(2.58)	84.80(8.38)	81.04(6.64)	84.32(6.69)
10 min	79.06(3.17)	84.48(6.07)	73.08(6.09)	78.16(9.35)
60 min	82.46(7.70)	83.84(6.34)	81.26(2.41)	85.24(8.32)
120 min	80.92(2.72)	81.96(1.88)	79.78(7.48)	85.10(6.73)
180 min	81.58(3.88)	74.68(24.93)	83.50(4.28)	83.50(4.07)

Table 11.2: Venous pO_2 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous pO_2			
	Plac	Pbz	Rom	Pbz_Rom
0 min	33.5(4.81)	38.08(7.57)	35.80(6.48)	38.82(3.18)
10 min	33.92(5.73)	38.42(6.75)	25.12(3.21)	30.60(4.23)
60 min	30.46(4.21)	32.66(3.37)	29.08(4.36)	34.34(2.51)
120 min	33.74(2.86)	32.36(3.18)	30.72(5.48)	30.94(4.85)
180 min	36.38(6.76)	47.36(26.9)	26.42(2.68)	31.44(3.56)

Table 12.1: Arterial Na^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial Sodium			
	Plac	Pbz	Rom	Pbz_Rom
0 min	138.8(3.70)	135.2(2.77)	137.0(3.54)	137.0(4.69)
10 min	137.2(3.96)	135.2(2.68)	136.0(1.00)	138.6(5.50)
60 min	137.2(3.70)	135.0(2.74)	136.0(1.87)	136.0(1.58)
120 min	137.6(3.21)	135.8(2.05)	135.8(0.84)	136.4(1.67)
180 min	137.2(3.03)	135.4(2.51)	136.2(0.84)	136.0(1.22)

Table 12.2: Venous Na^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous Sodium			
	Plac	Pbz	Rom	Pbz_Rom
0 min	137.4(3.85)	135.6(2.88)	136.2(1.30)	137.8(5.26)
10 min	137.6(3.65)	135.4(2.97)	136.2(1.30)	138.8(5.72)
60 min	137.6(3.36)	135.8(2.49)	135.8(1.64)	136.4(2.07)
120 min	137.4(2.88)	136.4(2.07)	136.6(0.89)	137.0(1.87)
180 min	137.4(2.51)	136.2(2.28)	137.2(1.30)	136.6(1.52)

Table 13.1: Arterial K^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80µg /kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial Potassium			
	Plac	Pbz	Rom	Pbz_Rom
0 min	3.932(0.22)	4.148(0.25)	3.944(0.18)	4.156(0.20)
10 min	3.868(0.24)	4.090(0.29)	3.884(0.17)	4.182(0.30)
60 min	3.778(0.24)	3.962(0.42)	3.888(0.06)	3.826(0.15)
120 min	3.898(0.19)	3.842(0.32)	3.920(0.21)	3.856(0.24)
180 min	3.876(0.25)	3.850(0.34)	3.670(0.08)	3.598(0.13)

Table 13.2: Venous K^+ of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous Potassium			
	Plac	Pbz	Rom	Pbz_Rom
0 min	3.940(0.16)	4.140(0.23)	3.982(0.22)	4.216(0.19)
10 min	3.918(0.17)	4.100(0.29)	3.924(0.17)	4.340(0.13)
60 min	3.750(0.22)	4.022(0.46)	3.914(0.12)	3.836(0.12)
120 min	3.932(0.20)	3.872(0.33)	3.944(0.13)	3.894(0.23)
180 min	3.920(0.26)	3.870(0.33)	3.810(0.08)	3.712(0.19)

Table 14.1: Arterial Ca^{2+} of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial Calcium			
	Plac	Pbz	Rom	Pbz_Rom
0 min	1.636(0.11)	1.638(0.11)	1.570(0.09)	1.616(0.11)
10 min	1.674(0.21)	1.652(0.17)	1.554(0.08)	1.616(0.14)
60 min	1.586(0.12)	1.596(0.14)	1.472(0.05)	1.474(0.05)
120 min	1.650(0.11)	1.594(0.09)	1.514(0.06)	1.492(0.07)
180 min	1.638(0.11)	1.594(0.09)	1.546(0.09)	1.460(0.09)

Table 14.2: Venous Ca^{2+} of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	**Venous Calcium**			
	Plac	**Pbz**	**Rom**	**Pbz_Rom**
0 min	1.660(0.16)	1.672(0.18)	1.574(0.05)	1.648(0.16)
10 min	1.682(0.21)	1.700(0.23)	1.562(0.06)	1.624(0.16)
60 min	1.610(0.07)	1.618(0.19)	1.492(0.04)	1.486(0.06)
120 min	1.638(0.15)	1.632(0.15)	1.532(0.07)	1.504(0.08)
180 min	1.648(0.13)	1.662(0.18)	1.554(0.07)	1.478(0.07)

Table 15.1: Arterial HCO_3 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial HCO_3			
	Plac	Pbz	Rom	Pbz_Rom
0 min	25.16(1.24)	25.44(1.12)	25.56(1.98)	23.76(1.86)
10 min	26.10(1.20)	25.76(1.29)	26.50(1.77)	25.12(1.71)
60 min	25.78(1.19)	25.48(1.56)	27.04(2.04)	26.24(1.60)
120 min	25.86(0.74)	25.18(0.97)	27.40(2.24)	26.80(1.45)
180 min	25.58(0.49)	25.60(1.69)	27.30(1.80)	26.42(1.64)

Table 15.2: Venous HCO_3 of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous HCO_3			
	Plac	Pbz	Rom	Pbz_Rom
0 min	25.72(0.50)	25.26(0.65)	25.64(2.21)	24.94(1.00)
10 min	25.82(1.15)	25.52(1.46)	25.98(2.10)	24.74(1.63)
60 min	26.44(1.48)	25.62(1.78)	25.96(2.39)	26.34(1.25)
120 min	25.66(1.83)	25.52(1.24)	27.16(1.90)	26.58(1.61)
180 min	25.72(1.09)	25.84(1.05)	27.12(2.74)	26.20(1.06)

Table 16.1: Arterial BE of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Arterial BE			
	Plac	Pbz	Rom	Pbz_Rom
0 min	0.66(1.37)	0.84(1.37)	1.06(2.62)	-1.38(2.76)
10 min	1.90(1.32)	1.34(1.60)	2.60(2.15)	0.70(2.29)
60 min	1.56(1.54)	1.04(1.98)	3.18(2.77)	2.24(2.04)
120 min	1.70(0.93)	0.62(1.00)	3.70(2.72)	2.82(1.82)
180 min	1.20(0.51)	1.52(1.61)	3.66(2.15)	2.50(1.98)

Table 16.2: Venous BE of five horses administered saline iv (Plac), phenylbutazone (4.4 mg/kg iv) (Pbz), romifidine (80 µg/kg iv) (Rom) and a combination of phenylbutazone and romifidine (Pbz_Rom). Mean(SD).

Time	Venous BE			
	Plac	Pbz	Rom	Pbz_Rom
0 min	2.78(0.44)	1.76(0.57)	2.48(3.01)	1.22(1.04)
10 min	2.88(1.27)	2.10(1.56)	3.88(2.44)	1.88(1.78)
60 min	4.00(1.44)	2.72(2.04)	3.50(3.12)	3.60(1.51)
120 min	2.54(2.64)	2.56(1.43)	5.00(2.37)	4.12(1.69)
180 min	2.46(1.25)	2.50(1.87)	5.38(3.54)	3.66(1.11)

APPENDIX B

Unforeseen events that occurred in the research population were as follows:

One horse was found dead in his paddock four weeks after superficial translocation of his left carotid artery. He had bled out from that carotid artery. We suspect that he must have sustained trauma to the superficial artery leading to the unfortunate incident.

One horse developed a severe encephalopathy during the second week of the trial. It had received the placebo treatment four days prior to developing clinical signs. This horse was euthanized on humane grounds due to severe neurological derangements leading to self-trauma.

Three horses were suspected to be infected with Equine Encephalosis Virus during the trial. One of these horses developed Piroplasmosis subsequent to the viral infection. These three horses were treated successfully. They were temporarily withdrawn from the trial until all clinical and clinicopathological parameters had returned to normal, where after they returned.