Coagulation, oncotic and haemodilutional effects of a third generation hydroxyethyl starch (130/0.4) in ponies

By

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Submitted in partial fulfilment of the requirements for the MMedVet degree in the Faculty of Veterinary Science, University of Pretoria, South Africa

Table of Contents

Dedication

To everything there is a season:

There is a time for everything,

And a season for every activity under the heaven:

A time to be born and a time to die,

A time to plant and a time to uproot;

A time to kill and a time to heal,

A time to tear down and a time to build;

A time to weep and a time to laugh,

A time to mourn and a time to dance.

Ecclesiastes 3: 1-4

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List of Abbreviations

PCV	Packed cell volume
TS	Plasma total solids
COP	Colloid osmotic pressure
TEG	Thromboelastography
HES	Hydroxyethyl starch
MW	Molecular weight
kiloDalton	kDa
MS	Molar substitution
R-time	Reaction time
α-angle	Alpha angle
MA	Maximum amplitude
ND	No data
NA	Not available
Cr	Creatinine
SBA	Serum bile acids

List of Figures

List of Tables

Summary

COAGULATION, ONCOTIC AND HAEMODILUTIONAL EFFECTS OF A THIRD GENERATION HYDROXYETHYL STARCH (130/0.4) IN PONIES

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This dissertation describes the effects of tetrastarch (130/0.4) on serum colloid osmotic pressure and thromboelastography variables in healthy pony mares. Additional variables assessed during this study included markers of haemodilution (PCV, TS) and serum creatinine and bile acid concentrations.

Six clinically healthy Nooitgedacht pony mares were utilized in a crossover study design. Tetrastarch (130/0.4) was administered at 10, 20 and 40 ml/kg bwt to each mare in a random sequence with a two week washout period between each of the treatments. Packed cell volume (PCV), plasma total solids (TS), serum colloid osmotic pressure (COP), and platelet count were measured and thromboelastography (TEG) was performed before treatment (baseline), immediately after infusion (time 0), and 1, 6, 12, 24, 48, and 96 h after tetrastarch infusion.

All TEG variables remained within reference range in all treatment groups. Administration of tetrastarch at 40 ml/kg bwt resulted in a prolonged K-time at 6 h post-infusion, and decreased maximum amplitude at 0, 1, 6, 24 and 48 h post-infusion compared to baseline. Administration of tetrastarch increased mean COP values above baseline in all three treatment groups, persisting to 24, 6 and 48 h after treatment with 10, 20 and 40 ml/kg of tetrastarch respectively.

This study concluded that, although values remained within established reference ranges, the administration of tetrastarch (130/0.4) at 40 ml/kg bwt is more likely to induce changes in TEG variables than doses of 20 ml/kg or less. Tetrastarch increased COP in healthy horses at all evaluated dose rates.

Chapter 1: Literature review

Collectively, these data suggest that tetrastarch (130/0.4) administered at 10 and 20 ml/kg bwt has potential as a synthetic colloid for resuscitation and provision of oncotic support in horses. The results reported in this dissertation provide a reference for tetrastarch administration in horses. Due to current concerns regarding the safety of older hydroxyethyl starch formulations in humans, further investigation of the effects of hydroxyethyl starch in diseased horses is needed.

Key words: *horse; hydroxyethyl starch; tetrastarch; thromboelastography; oncotic pressure*

1.1 COLLOID ADMINISTRATION

The following literature review is based on literature available at the time that this study protocol was designed and performed, prior to recent concerns regarding the administration of hydroxyethyl starch (HES) as a resuscitative fluid.

1.1.1 General introduction to fluid resuscitation

Maintaining blood volume and tissue perfusion during major surgery and periods of hypovolaemia is of extreme importance, and intravenous fluid therapy remains the cornerstone of critical care medicine to maintain or restore intravascular blood volume [1]. Controversy still exists regarding the optimal choice of fluid for resuscitation. Crystalloid preparations provide both intravascular and interstitial fluid replacement, have minimal impact on coagulation, and are inexpensive and widely available [1]. One of the significant disadvantages of crystalloid preparations however, is the limited duration of intravascular volume expansion and subsequent distribution of excessive fluid into the interstitial space [2]. Sodium, the principle electrolyte in crystalloids, is also the main electrolyte within the extracellular fluid

Because the majority of extracellular fluid is within the interstitial compartment, crystalloids will distribute evenly throughout the extracellular fluid into the interstitial fluid space, which can result in interstitial oedema formation [2].

Colloid fluids have the advantages of prolonged intravascular habitation and smaller volume requirements compared to crystalloids [1]. Disadvantages attributed to colloid administration have been described extensively in the human medical literature and include allergic reactions, impairment of renal function and coagulation, as well as substantially greater expense. Despite the disadvantages associated with colloid therapy, the benefits of their administration have led to widespread use of colloids as plasma volume expanders in critically-ill patients around the world [1].

Large volume crystalloid administration can decrease oncotic pressure and increase hydrostatic pressure. Colloids increase oncotic pressure and are therefore deemed preferable when significant volume expansion is required. It is largely because of their beneficial effect on plasma oncotic pressure that colloids have been used extensively in veterinary medicine [1]. However, a paucity of literature exists regarding colloid administration in veterinary patients and current recommendations are mostly extrapolated from human medical literature. Colloid administration has also been described in the equine literature with potential benefits described in hypoproteinaemic horses and horses with colic and colitis [3,4]. However, recommendations regarding the maximum dose that can be administered without adversely affecting coagulation are extrapolated from human literature. Newer synthetic colloid preparations have been shown to have a less substantial impact on

coagulation in humans, however no data currently exists describing the potential advantages and adverse effects of these newer preparations in horses.

1.1.2 Hydroxyethyl starch solutions

Hydroxyethyl starches (HES) are synthetic colloids frequently used to expand plasma volume and to provide haemodynamic stabilization and improved tissue perfusion [5]. These starches are produced by hydrolysis and subsequent hydroxyethylation substitution of amylopectin, a branched polysaccharide polymer [6]. HES was first introduced in the 1970s, and since then, 2nd and 3rd generation HES have been developed, which differ in their mean molecular weight (MW) expressed in kiloDaltons (kDa), and in their molar substitution (MS) and C_2/C_6 ratio [7]. Three numbers representing three variables highly relevant to the pharmacokinetics of the different products identify HES. The first number represents the concentration of the solution as a percentage, the second number the MW, and the third number the MS *eg.* 6%, 130/0.4. These three characteristics together with the pattern of hydroxyethyl substitution determine the plasma expanding capacity of each HES product.

1.1.3 The chemistry of hydroxyethyl starch solutions

Hydroxyethyl starch is a derivative of waxy corn starch which mainly consists of a glucose polymer (amylopectin). It is this natural amylopectin that is modified by hydroxyethylation at the glucose subunit atoms C_2, C_3 or C_6 to produce HES molecules. HES can be classified according to mean MW as high-, medium-, or low-MW solutions, or according to MS as high, medium or low substitution solutions.

Additionally, they can be classified according to C_2/C_6 ratio into solutions with a high and low C_2/C_6 ratio [5].

HES are polydisperse solutions containing particles with a wide range of molecular mass and size. The mean MW can be described as either the weight averaged or the number averaged MW. Weight averaged MW is influenced by larger molecules and gives a larger value to the mean MW compared to number averaged MW. Following intravenous administration, small molecules below the renal threshold are readily and rapidly excreted while molecules with higher molecular weights are retained and metabolised by alpha-amylase prior to excretion. The duration for which these larger molecules are retained depends on the molecular size and ease of degradation. The osmotic effectiveness of HES depends on the number of molecules retained rather than the molecular size [7]. Thus low MW products with a higher number of molecules in a given volume of HES solution will exert a greater osmotic effect compared to high MW products.

Molar substitution, in effect, is the number of hydroxyethyl residues per glucose subunit within the amylopectin polymer. HES preparations with a MS of 0.7 have 7 hydroxyethyl residues per 10 glucose subunits. HES with this degree of MS are called hetastarches, while HES preparations with a MS of 0.6, 0.5 and 0.4 are called hexastarch, pentastarch and tetrastarch respectively. Unsubstituted glucose subunits are more prone to enzymatic degradation by alpha-amylase. Hydroxyethylation therefore prolongs intravascular retention time by increasing the solubility of HES in water and inhibiting the rate of degradation by alpha-amylase [7].

Apart from the MS, the pattern of hydroxyethylation (C_2/C_6 ratio) also plays a significant part in the pharmacokinetic properties of HES solutions. Hydroxyethylation of the glucose subunits predominantly occurs at the C_2 and C_6 carbon atoms. Access of alpha-amylase to its enzymatic cleavage site is inhibited more effectively by hydroxyethyl groups at the position of the C_2 atom compared to hydroxyethyl groups positioned at the C_6 carbon atom [8]. HES solutions with a higher C_2/C_6 ratio are therefore expected to decrease hydrolysis by alpha-amylase more effectively compared to HES with a similar MW and MS but lower C_2/C_6 ratio. Several studies have demonstrated how structural differences of HES solutions affect the pharmacokinetic properties of these substances [9,10].

1.1.4 Hydroxyethyl starches: effects on coagulation

Adverse effects of HES on haemostasis pose limitations on their clinical use, with the most pronounced effects associated with large and highly substituted HES molecules [5]. Specific adverse effects on coagulation include decreased circulating factor VIII and von Willebrand factor (vWF) concentrations, impairment of platelet function and decreased fibrin clot stabilization [5]. The pathogenic mechanism responsible for the adverse effects of HES on coagulation is poorly understood. Passive haemodilutional effects such as decreased fibrinogen and haemoglobin concentrations are observed immediately following administration while vWF reaches its nadir 1 to 2 hours following administration [5]. This suggests that additional mechanisms are responsible for impaired coagulation, beyond dilutional effects. HES molecules also interact with platelets, and it is speculated that HES molecules either attach to platelets or are phagocytosed by them. Therefore newer 3rd generation HES solutions

have been developed to reduce the risk of coagulopathies associated with HES administration [7].

1.2 TETRASTARCH 130/0.4

1.2.1 Pharmacokinetics

Tetrastarch 130/0.4 is a novel 3rd generation HES [7]. It has an average molecular weight of 130 kDa and MS of 0.4 to enhance degradation and limit potential side effects. It has been shown that tetrastarch 130/0.4 has a volume effect of approximately 100% and the duration of this increased plasma volume is approximately 4 to 6 hours in humans [11].

Tetrastarch 130/0.4 does not accumulate in plasma following multiple dosing, and renal excretion of tetrastarch 130/0.4 is higher than earlier HES formulations [12]. These properties result in reduced tissue storage of tetrastarch 130/0.4 compared to other HES. Hydroxyethyl starch solutions with a higher molecular weight tend to be stored in tissue before being metabolized by amylases. Tissue deposition appears to be dose-dependant but does not appear to impair organ function. [13].

1.2.2 Effects on coagulation and platelet function

A number of studies have investigated both the *in vivo* and *in vitro* effects of HES products on coagulation and platelet function, and have found that the newer generation HES products interfere significantly less with coagulation compared to older products [14, 15]. Variables investigated in these studies include coagulation factor VIII and von Willebrand factor, while platelet count and function have also been investigated. Overall, these studies suggest that newer HES products interfere significantly less with coagulation. Several studies report the potential effects of waxy maize-derived HES 130/0.4 on coagulation and confirm that tetrastarches have minimal effects on coagulation [11, 16, 17]. Only one report currently exists describing the *in vitro* effects of tetrastarch on coagulation of equine blood [18].

1.2.3 Effects on renal function:

This brief review of the impact of HES on renal function is based on the literature available at the time that this study protocol was designed. Earlier reports raised concerns about the possible deleterious effects of HES on renal function, but current data in human medicine suggests that these concerns should not be extrapolated to the newer generation HES preparations [19,20]. Earlier reports have also suffered from relatively short follow-up periods of assessment of renal function in treated patients [19]. In a 60-day follow-up study in humans undergoing cardiac surgery, kidney function in patients receiving tetrastarch was reportedly not different to those who received albumin [19].

The most current contribution to the debate about HES and renal function at the time that this study was performed was a study evaluating patients with severe sepsis [21]. The study investigated the influence of pentastarch (10% 200/0.5) *versus* crystalloid fluid resuscitation. The authors of the study reported that pentastarch administration was associated with a higher rate of acute renal failure and potential need for renal replacement therapy as compared to Ringer's lactate solution [21]. Over 38% of patients included in the study received significantly more than the maximum specified dose of pentastarch (20ml/kg/day). However, concurrent insulin therapy in this study may have contributed to acute renal failure, and was not evaluated, representing an important limitation of the study.

1.3 BRIEF LITERATURE REVIEW ON RECENT CONCERNS ASSOCIATED WITH COLLOID ADMINISTRATION:

The following literature review includes recent concerns regarding HES administration that were published after the study protocol of this thesis was approved and conducted.

1.3.1 Introduction

The use of HES for fluid resuscitation is controversial, and concerns regarding the safety of such solutions have been raised repeatedly. Large clinical trials and retrospective analyses have highlighted the effect of HES on renal function, with evidence suggesting that patients treated with HES are at increased risk of renal dysfunction [22-26]. However, in contrast, it has been shown that HES administration improves outcome in intensive care departments and have been widely used in certain countries [27-29]. Concerns regarding HES associated renal dysfunction have progressively increased, and clinical trials were developed to evaluate the efficacy and safety of the newer generation HES solutions such as HES 130/0.4 and HES 130/0.42. These concerns increased after the retraction of clinical trials performed by an investigator due to scientific misconduct [26]. In 2011, 86% of the research published by the investigator in question was retracted after reported failure to acquire ethical research approval and fabrication of data. As a result of these retractions, all major systematic reviews on HES and renal dysfunction were revised to account for retracted data.

1.3.2 Publications evaluating renal dysfunction and HES administration:

Hydroxyethyl starch or saline for fluid resuscitation in intensive care [23]

In 2010, the crystalloid versus HES trial (CHEST) was initiated to further investigate the safety of HES solutions in intensive care patients. This protocol was designed as a multicentre randomised controlled clinical trial to study the effect of fluid resuscitation with 6% HES (130/0.4) compared to 0.9% sodium chloride (saline) on mortality in intensive care patients. The study included patients who were 18 years or older and whom the clinicians had judged to require fluid resuscitation. All patients with impending or current dialysis-dependant renal failure were excluded. Patients received a maximum daily dose of HES of 50 ml/kg. The results of this multicentre trial were published in 2012 and concluded that resuscitation with HES resulted in a higher incidence of kidney injury compared to saline, however, no difference in 90-day mortality could be detected between patients resuscitated with HES or saline. The study concluded that resuscitation with HES, as compared with saline, does not provide any clinical benefit to the patient and that the potential for renal dysfunction should be considered carefully prior to selection of an appropriate resuscitation fluid.

Hydroxyethyl starch 130/0.42 versus ringer's acetate in severe sepsis [24]

The Scandinavian starch for severe sepsis/septic shock (6S) trial was conducted to evaluate the effects of HES 130/0.42 (Tetraspan 6%) compared to Ringer's acetate on mortality and end-stage kidney failure in patients with severe sepsis. Patients included in the trial were 18 years and older, who needed fluid resuscitation, and who fulfilled the criteria for severe sepsis within the previous 24 hours. The study concluded that patients with severe sepsis who received fluid resuscitation with HES compared to Ringer's acetate were at increased risk of mortality by day 90 and were more likely to require renal replacement therapy. Patients included in this trial received a maximum daily dose of 33 ml/kg bwt of tetraspan, and if doses higher than the maximum recommended daily dose of tetraspan were required, Ringer's acetate was used regardless of the patient's treatment assignment. This study did have the limitation that patients with acute kidney injury were included in the trial, however, their inclusion was not likely to have affected the outcome of the trial because kidney injury occurred with equal frequency in the two intervention groups, and the effect of HES on mortality and renal dysfunction was not found to differ significantly between patients with and without renal disease.

'Fluid resuscitation with 6% hydroxyethyl starch (130/0.4 and 130/0.42) in acutely ill patients: systematic review of effects on mortality and treatment with renal replacement therapy' [25]

This article describes 35 clinical trials that were evaluated to compare the effect of HES (6%, 130/0.4 or 130/0.42) to other resuscitative fluids. It was concluded that patients randomly assigned to resuscitation with HES were at increased risk of requiring renal replacement therapy.

'Association of hydroxyethyl starch administration with mortality and acute kidney injury in critically ill patients requiring volume resuscitation' [26]

This article represents a systematic review and meta-analysis evaluating the association of HES with mortality and acute kidney injury in human patients. It included 38 eligible trials comparing different HES formulations to crystalloids, albumin or gelatin. The study concluded that the use of HES as a resuscitation fluid was not associated with reduced mortality. After exclusion of 7 trials performed by an investigator guilty of scientific misconduct, HES was actually found to be associated with an increased risk of mortality and acute kidney injury.

In conclusion, current literature raises concerns regarding the safety of HES and suggests that some HES formulations are more likely to increase the risk of acute renal injury and the need for renal replacement therapy in human patients. Based on these concerns, it is currently recommended that alternate replacement therapies be used in place of HES solutions in humans until a safe volume and formulation of HES solution has been determined.

1.4 MONITORING OF COAGULATION

The haemostatic process of coagulation is extremely complex and involves multiple interactions between platelets and coagulation factors, activators and inhibitors. As technology progresses, evaluation of haemostasis will become more specific and accurate, which will facilitate accurate monitoring of haemostasis in the clinical setting.

1.4.1 Standard tests of coagulation

In order to appropriately direct therapy, it is important that the underlying cause of any medical coagulopathy be determined. Several disease processes including colic, disseminated intravascular coagulation and sepsis are associated with coagulation abnormalities in horses [30,31]. Traditional methods for evaluation of coagulation require interpretation of information obtained from a variety of assays, each assessing individual aspects of the coagulation cascade. Clinicopathological tests previously used to describe coagulation in horses include coagulation times (prothrombin time [PT], activated partial thromboplastin [aPTT] time and thrombin time) and platelet number and aggregation [32]. Other clinicopathological tests that are available evaluate specific anticoagulant factors, and factors associated with inhibition of fibrinolysis [32]. Interpretation of the aforementioned tests may be difficult and whole blood dynamic tests such as thromboelastography (TEG) have been developed to provide rapid and comprehensive assessment of clot formation rather than measuring end-points of coagulation.

1.5 THROMBOELASTOGRAPHY

1.5.1 Introduction

Thromboelastography is a viscoelastic, non-invasive whole blood based assay designed to analyse both cellular and soluble components of the coagulation process. It provides a more global assessment of the equine haemostatic system by detecting changes in the viscoelastic properties of a blood clot from its formation to fibrinolysis [33]. Thus, in essence, TEG measures the ability of a clot to perform mechanical work throughout its structural development. A significant advantage of TEG over conventional standard plasma-based assays is that it allows for identification of either hypo- or hypercoagulability in whole blood samples. It assesses whole blood coagulation rather than individual components of the coagulation pathway. Different techniques can be used to perform TEG assays. When the assay is performed without an activator, the technique is called native citrated TEG. This technique has the disadvantage of a high variability. Thromboelastography assays can also be performed with an activator such as kaolin, which stimulates the intrinsic pathway, or human recombinant tissue factor, which stimulates the extrinsic pathway. The use of activators minimises analytical and user variation [32].

1.5.2 Thromboelastography assessment

Thromboelastography is a graphical method of displaying the kinetics of clot formation and dissolution, as well as clot quality. Hartert first developed the technique in 1948 [34]. The TEG graph is divided into several components or variables.

The TEG variables commonly measured in horses include the following:

Reaction time (R-time): is the period of time elapsing from when blood is placed in the TEG analyser until detectable clot formation occurs. It is correlated with the activity of plasma coagulation factors. Reaction time can be shortened by hypercoagulable conditions such as disseminated intravascular coagulation, and prolonged by factor deficiencies and anticoagulant agents.

K-time: this represents a measure of the speed of clot formation and is typically characterised as the time from the end of R measurement until a set clot strength is achieved. K-time is related to clot kinetics and corresponds to the initial activation of platelets and fibrinogen. It can be shortened by increased fibrinogen concentrations and prolonged by anticoagulants and hypofibrinogenaemia.

Alpha angle (α-angle): is closely related to K-time and corresponds to the slope of the tangent on the elasticity curve and indicates a tendency towards hypo- or hypercoagulable conditions. It measures the rapidity of clot strengthening.

Maximum amplitude (MA): represents the overall clot strength and depends on the contribution of two components: primarily platelet aggregation, and a modest contribution of fibrin to clot strength.

G-value: Is calculated using the MA and represents the viscoelastic strength of the clot.

LY30 and LY60: Provide evidence of thrombolysis. It is a measure of amplitude reduction 30 and 60 minutes after MA respectively and is measured as a percent decrease in area under the curve at 30 and 60 minutes compared with MA.

In general, prolonged R- and K-times together with decreased MA and angle values compared to normal are characteristic of hypocoagulability. The opposite is true for hypercoagulability that is characterised by decreased R- and K-times and increased MA and angle values compared to normal. In general, TEG interpretation requires minor training and the most relevant values including R- and K-time, MA and angle values are available for interpretation within 30 minutes [35].

1.5.3 Thromboelastography in the horse

Research regarding TEG and its interpretation is limited in horses. Reported TEG values for horses are summarised in tables 1.1 and 1.2 respectively. A wide range of TEG reference values exist in the equine literature, a phenomenon attributed to high inter-individual variability, and differences in storage times, operators, blood collection techniques and TEG techniques [35]. Maximum amplitude appears to have the lowest variability amongst the TEG variables and may therefore be the most reliable of the TEG variables in horses [36]. Due to the high variability in published TEG values for healthy horses, it is important that each laboratory evaluate a group of healthy horses in order to establish their own in-house reference intervals.

1.5.3.1 Clinical application of thromboelastography in horses

Hypercoagulation is a state characterised by more rapid and stronger clot formation. Historically this state has been recognised in horses with early inflammatory or ischaemic lesions. With disease progression, fibrin and thrombus formation occurs throughout the microvasculature resulting in DIC. Hypocoagulation can be expected in horses with severe gastrointestinal disease, sepsis, hepatic disease, or inherited haemorrhagic disorders such as vitamin K deficiency.

'Association between hypercoagulability and decreased survival in horses with ischemic or inflammatory gastrointestinal disease' [37]

Hypercoagulability was assessed by TEG using sodium citrate blood samples from horses. TEG profiles from horses with ischaemic or inflammatory GI disease were compared with profiles from horses with non ischaemic or inflammatory GI disease and healthy horses. This study indicated that horses with ischaemic or inflammatory gastrointestinal disease had decreased R times compared to normal horses. The authors concluded that although evidence of hypercoagulability exists in horses with gastrointestinal disease, techniques for accurate diagnosis of this phenomenon required refinement, as TEG was only able to establish a tendency towards hypercoagulability. One of the major limitations of this study included a significant overlap in the various parameters measured between healthy and affected horses. TEG profiles obtained form healthy horses varied significantly, and the authors commented that the TEG technique used in the study is of limited clinical use and treatment decisions should not be based on TEG findings alone.

'Thromboelastography in horses with acute gastrointestinal disease' [38]

In this study, TEG was used to identify coagulation abnormalities associated with lesion type, the presence of systemic inflammatory response syndrome, and morbidity and fatality. Thromboelastography was performed with and without tissue factor activation and it was concluded that both techniques were able to identify changes in coagulation and fibrinolysis indicative of hypocoagulation and hypofibrinolysis.

'Thromboelastography in healthy horses and horses with inflammatory gastrointestinal disorders and suspected coagulopathies' [36]

This study evaluated the use of citrated nonactivated TEG in healthy horses in comparison with a group of horses with colitis and prolonged clotting times. Horses were only included in the study if they had a clinical diagnosis of colitis and prolonged clotting times as measured by PT and/or aPTT). Thromboelastography changes that were detected were consistent with hypocoagulability in horses with inflammatory gastrointestinal disorders and a previously determined coagulopathy. The authors commented that a larger number of cases and different stages of severity would be needed to establish the clinical utility of TEG and to determine whether TEG could replace traditional coagulation testing.

Sonoclot and thromboelastometry are two other whole blood dynamic tests of coagulation that have been validated in horses [31,39]. As with TEG, these dynamic blood tests take into account the function of both cellular and soluble components of coagulation evaluating the efficiency of the whole coagulation process.

In conclusion, further prospective clinical studies are needed to determine whether TEG can replace conventional coagulation testing for detecting hypo- or hypercoagulability in horses. However, based on clinical studies performed thus far, TEG may be a valuable addition to procedures assessing the haemostatic profile of horses, assisting clinicians in characterising the entire coagulation process and in monitoring response to treatment.

1.6 Figures and tables

Table 1.1: Reference intervals (mean ± s.d.) of citrate, non-activated thromboelastography in healthy horses.

Study and reference number	Year	Adults	R-time (min)	K-time (min)	Angle (degrees)	MA (mm)	LY30 (min)	LY60 (min)
Paltrinieri *et al.* [31]	2008	Adults	8.1 ± 4.7	3.0 ± 1.4	59.8 ± 10.9	50.0 ± 8.1	ND	ND
Epstein *et al.* [32]	2009	Adults	17.0 ± 3.0	5.8 ± 2.3	42.0 ± 14	60.3 ± 5.7	0.8 ± 0.6	3.2 ± 2.5
Leclere *et al.* [40]	2009	Adults	16.6 ± NA	6.05 ± NA	30.5 ± NA	42.3 ± NA	ND	ND
Mendez-Angulo *et al.* [36]	2010	Adults	10.4 ± 3.1	3.5 ± 1.2	55.6 ± 11.0	55.6 ± 5.1	1.0 ± 0.8	3.4 ± 2.3
Dunkel *et al.* [37]	2010	Adults	22.8 ± 12	13.0 ± 19.5	30.6 ± 17.0	57.3 ± 14.0	1.0 ± 0.8	3.4 ± 2.3
Epstein *et al.* [38]	2011	Adults	16.2 ± 5.8	5.7 ± 3.7	42.5 ± 13.2	61.7 ± 8.4	0.7 ± 0.7	3.8 ± 1.7

No data: ND; Not available: NA

Table 1.2: Reference intervals (mean ± s.d.) of tissue factor activated thromboelastography in healthy horses.

Study and reference number	Year	Adults	R-time (min)	K-time (min)	Angle (degrees)	MA (mm)	LY30 (min)	LY60 (min)
Epstein *et al.* [32]	2009	Adults	6.6 ± 1.4	3.1 ± 1.0	50.9 ± 9.0	62.3 ± 5.1	0.6 ± 0.5	3.6 ± 1.9
Leclere *et al.* [40]	2009	Adults	5.0 ± NA	3.6 ± NA	43.6 ± NA	53.1 ± NA	ND	ND
Epstein *et al.* [38]	2011	Adults	6.9 ± 1.3	3.2 ± 1.1	52.8 ± 7.5	62.9 ± 5.2	0.5 ± 0.5	3.0 ± 1.5

No data: ND; Not available: NA

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Coagulation, oncotic and haemodilutional effects of a third generation hydroxyethyl starch (130/0.4) in ponies

2.1 ABSTRACT

Clinical indications for hydroxyethyl starches (HES) in horses include the need for rapid plasma volume expansion and oncotic support during periods of shock and/or hypoproteinaemia. Side effects such as coagulopathy potentially pose limitations to their use in veterinary medicine. In humans, tetrastarch has demonstrated less profound effects on coagulation compared to first and second generation HES products.

The objective of this study was therefore to evaluate the haemostatic and oncotic effects of tetrastarch (130/0.4) administered at 10, 20 and 40 ml/kg bwt in healthy ponies. We hypothesized that tetrastarch administered at 10 and 20 ml/kg would not be associated with adverse effects on coagulation, and that tetrastarch would significantly increase serum colloid osmotic pressure (COP) at all doses studied.

Tetrastarch (130/0.4) was administered to 6 healthy pony mares at 10, 20 and 40 ml/kg bwt in a random sequence using a crossover design with a 2-week washout period between treatments. Packed cell volume (PCV), plasma total solids (TS), serum COP, platelet count and thromboelastography (TEG) variables were measured before treatment (baseline), immediately after treatment (time 0), and 1, 6, 12, 24, 48, and 96 hours after tetrastarch infusion.

All TEG variables remained within reference range in all treatment groups. However, administration of tetrastarch at 40 ml/kg bwt resulted in a longer K-time (P=0.049) at 6 h post-infusion, and decreased maximum amplitude at 0 (P<0.001), 1 (P=0.022), 6 (P=0.006), 24 (P<0.001) and 48 h (P=0.013) post-infusion compared to baseline. Administration of tetrastarch significantly increased mean COP above baseline in all treatment groups, persisting for 24, 6 and 48 h for the 10, 20 and 40 ml/kg bwt doses respectively.

Although TEG variables remained within established reference ranges, compared to lower doses the administration of 40 ml/kg bwt tetrastarch (130/0.4) is more likely to induce changes in coagulation as measured by TEG. Tetrastarch increased COP at all doses evaluated in healthy horses.

Data suggest that tetrastarch (130/0.4) at 10 and 20 ml/kg bwt has potential as a synthetic colloid for resuscitation and provision of oncotic support in horses without adverse effects on coagulation.

2.2 INTRODUCTION

Hydroxyethyl starch (HES) preparations are artificial colloid solutions used for intravascular volume expansion and provision of plasma oncotic support during periods of hypoproteinaemia. Plasma volume expansion is achieved by increasing plasma colloid oncotic pressure via retention of large osmotically active molecules within the vasculature following intravenous (i.v.) administration [1].

Hydroxyethyl starches are produced by hydrolysis and subsequent hydroxyethylation substitution of amylopectin, a branched polysaccharide polymer [2]. Since the introduction of 1st generation HES (hetastarch), 2nd (pentastarch) and 3rd generation (tetrastarch) HES have been developed with differences in mean molecular weight (MW), degree of molar substitution (MS), C_2/C_6 ratio and concentrations [3]. These variables determine the pharmacokinetics of the product. Potential side effects, including coagulopathies, may be more frequent following the administration of 1st and 2nd generation HES solutions. Tetrastarch (130/0.4) is a novel 3rd generation HES with an average MW of 130 kDa, which is smaller than that of hetastarch, and MS of 0.4 to enhance degradation and limit potential side effects [4]. Preparations of tetrastarch can be derived from either maize or potato starch.

After administration, colloid molecules are more effectively restricted to the intravascular space, resulting in more rapid intravascular volume replacement and expansion compared to crystalloids that distribute among the extracellular fluid spaces [5,6]. Fluid resuscitation with crystalloids in horses with hypoproteinaemia may therefore lead to further dilution of plasma proteins and a decrease in oncotic pressure, contributing to oedema formation [7,8]. Intravenous administration of colloids have been found to maintain plasma oncotic pressure and plasma volume even when there is increased capillary permeability and albumin leakage [9]. Colloid solutions can therefore be used to restore and maintain oncotic pressure in horses affected by these conditions [8].

Despite potential advantages associated with HES administration, the use of synthetic colloids is limited by expense and possible adverse effects, including allergic reactions, coagulopathies, and recent reports of an increased risk of renal failure and mortality in critically-ill human patients [4,10,11]. Specific effects on coagulation include decreased circulating factor VIII and von Willebrand factor concentrations, impairment of platelet function and decreased fibrin clot stabilisation [4], which have been described in dogs and horses [1,12]. Other potential complications associated with colloid administration include haemodilutional effects causing decreases in measured serum total protein and albumin concentrations [1,12,13].

Many reports describe the potential benefits of colloid administration in horses [1,8,14], however, information regarding their oncotic and potential adverse effects is limited. The *in vitro* effects of HES solutions on coagulation and platelet function in horses have recently been described [15], and potential changes in coagulation following HES administration have been investigated *in vivo* [1,16]. Reported adverse effects are predominantly dose-related [1,15], and current recommendations for maximum daily tetrastarch dosage requirements in horses are mostly extrapolated from the human medical literature.

2.3 OBJECTIVES

This study was designed to determine the effects of tetrastarch (130/0.4) administration on coagulation variables and serum osmotic pressure in healthy ponies.

2.4 HYPOTHESES

We hypothesized that:

1. Tetrastarch administration at doses at or below 20 ml/kg would not be associated with changes in coagulation as measured by TEG in healthy ponies.
2. All administered dosages would increase serum colloid osmotic pressure (COP) in healthy ponies.

2.5 MATERIALS AND METHODS

2.5.1 Study design

Six clinically healthy, non-pregnant Nooitgedacht pony mares from the Onderstepoort Teaching Animal Unit were selected for the study. The selected ponies had a mean age of 5.3 years (range 2-10 years), and mean body mass of 422 kg (380–440 kg). Mares were considered clinically healthy on the basis of normal physical examination findings, normal complete blood count and serum biochemistry profiles, and normal haemostatic assays as determined by thromboelastography (TEG). All mares were hospitalised on Day 1 and 2 of each treatment protocol and received *ad libitum* grass (*Eragrostis tef*) and alfalfa (*Medicago sativa*) hay in addition to water. Thereafter they were housed in sheltered outside paddocks and were fed *ad libitum* hay with free access to water until study completion. The study was approved by the Faculty of Veterinary Science Research Committee and the University of Pretoria Institutional Animal Ethics Committee (protocol number V038/11).

2.5.2 Treatment administration

Treatments were randomly allocated to each mare using a prospective cross-over design. A 12-gauge, single lumen polyurethane intravenous catheter[a] was inserted into the left jugular vein and sutured in position. Catheters were only used for treatment administration and were removed following each infusion. Tetrastarch (130/0.4)[b] was administered intravenously to each mare once every two weeks at three doses: 10, 20, and 40 ml/kg bwt (Figure 2.1). Treatments were administered as an intravenous bolus by gravity using 500 ml tetrastarch bags. To maintain a constant flow rate, all bags were maintained at a pre-set height and were connected to a high flow (10 drops/ml) administration set[c] prior to colloid administration (Figure 2.2).

2.5.3 Blood sampling and analyses

Venipuncture of the right jugular vein was performed for the measurement of COP, packed cell volume (PCV), plasma total solids (TS), platelet count and TEG variables prior to colloid administration (baseline), immediately after each infusion (0 hours), and at 1, 6, 12, 24, 48, and 96 hours thereafter. Venous blood gas analyses were performed at baseline and at 0, 1, 6, 12 and 24 hours post-infusion. Serum bile acids (SBA) were measured at baseline and 0, 24, and 96 hours, while creatinine concentrations were measured at baseline and at 0, 6, 12, 24, 48, and 96 hours. Blood for PCV and TS analyses was collected into evacuated EDTA tubes[d]. Packed cell volume and TS were determined using microhaematocrit tubes and a refractometer. Blood was obtained into evacuated serum tubes[d] to measure serum bile acid and creatinine concentrations via automated serum biochemistry analyser[e] and COP via colloid osmometry[f]. Blood collected into an evacuated tube containing 3.2% sodium citrate[d] was used for TEG and platelet counts. Platelet counts were determined using an automated analyser[g]. Venous blood gas analyses were performed on blood collected into lithium heparin tubes[d]. The order of collection using a 21-gauge needle was as follow: serum, sodium citrate, EDTA followed by the lithium heparin sample.

2.5.4 Thromboelastography

Thromboelastography analyses were performed using the Thrombelastograph Hemostatic Analyser[i]. Machine balance and e-test (which measures the zero point) was verified prior to each analysis. The citrated blood sample was maintained at room temperature for 30 minutes prior to analysis. Twenty µL of 0.2M calcium chloride was added to a new preheated (37°C) reaction cup in one of the TEG channels. The blood in the citrated tube was mixed gently by inverting the tube 5 times prior to adding 1 ml of blood to the tube containing kaolin. The kaolin tube with added blood was then gently inverted 5 times to mix the kaolin and citrated whole blood and then 340 µL kaolin-activated citrated whole blood was added to each reaction cup. The following TEG variables were measured for each sample and compared with in-house laboratory reference ranges: reaction time [R (min), the time from when the blood sample is placed into the TEG cuvette until initial fibrin formation occurs]; coagulation time [K (min), the speed at which a clot forms with a certain viscoelastic strength]; angle [α (degrees), a measure of speed of clot formation]; maximal amplitude [MA (mm) a measure of clot strength]. In-house laboratory reference ranges were determined from 12 healthy Nooitgedacht pony mares. Mares were determined to be healthy based on clinical examination and haematological profiles. Reference ranges (minimum and maximum) were established by determining the mean ± 2 s.d. Two operators performed the TEG analyses and to minimise variability, TEG at baseline, and up to 6 hours after infusion were performed by the same operator.

2.5.5 Data analysis

Data were described using means, standard deviations, and calculating the change from baseline to 12 h post-infusion. Data were assessed for normality by evaluating descriptive statistics, plotting histograms and performing the Anderson-Darling normality test within statistical software[j]. Data that were not normally distributed were transformed using either the natural logarithm or by ranking prior to statistical analysis. R-time, K-time, G, LY30, COP, bicarbonate concentration (HCO_3^-) and calcium were not normally distributed and are described as median and interquartile range (IQR). A general linear modelling approach that incorporated adjustment for the repeated sampling of horses was used to analyse coagulation and blood parameter data. Linear mixed models were fit including fixed effect terms for tetrastarch dose, time of sampling, week of the study, and an interaction between dose and sampling time. A random effect for horse was included and a first order autoregressive correlation structure was used to adjust for repeated measurements. *Post hoc* comparisons were adjusted using the Bonferroni correction of *P* values. Statistical modelling was performed in commercially available software[k] and results interpreted at the 5% level of significance ($P<0.05$).

2.6 RESULTS

No adverse behavioural changes related to treatment were observed. Mean ± s.d. administration times for the 10, 20 and 40 ml/kg bwt dosages were 20.5 ± 1.64 min, 44.6 ± 10.57 min and 77.1 ± 7.96 min respectively.

2.6.1 Thromboelastography

Mean environmental temperatures at which TEG values were obtained were 24.62 (range 21.65-26.65), 23.25 (range 20.65-25.15) and 23.41 °C (range 20.15-25.15) for treatment weeks 1, 2 and 3 respectively. Thromboelastometric values at baseline were comparable with the established in-house laboratory reference ranges for kaolin-activated TEG (Table 2.1). Thromboelastometric results are summarised in tables 2.1 and 2.2 respectively.

Thromboelastometric values at baseline were comparable with the established in-house laboratory reference ranges for kaolin-activated TEG (Table 2.1 and Table 2.2). Overall R-time values differed significantly with tetrastarch dose ($P<0.001$), and the median (IQR) R-time change from baseline to 12 h was 3.0 (0.8 to 4.2), 1.3 (0.4 to 1.8), and 4.9 (2.1 to 10.9) minutes for the 10, 20 and 40 ml/kg bwt groups respectively. The 10 and 40 ml/kg bwt treatment groups ($P<0.001$) and the 20 and 40 ml/kg bwt treatment groups ($P=0.003$) were significantly different, but the 10 and 20 ml/kg bwt treatment groups were not ($P=1.0$). No significant changes over time were observed for R-time in any of the treatment groups.

Overall, K-time was not different among groups (P=0.697) and the median (IQR) K-time change from baseline to 12 h was 0 (-0.1 to 0.4), -0.1 (-0.4 to 0.1), and 1.4 (0.7 to 1.6) min for the 10, 20 and 40 ml/kg bwt groups respectively. Significant changes over time were not observed for K-time in the 10 and 20 ml/kg bwt groups, although a significant increase was observed at 6 h post-infusion of 40 ml/kg bwt (Table 2.1). In contrast, α-angle did not change significantly over time in any of the treatment groups, but there was an overall significant difference with tetrastarch dose (P<0.001). The mean (s.d.) α-angle change from baseline to 12 h was -3.05 (3.89), -1.90 (7.28), and -11.08 (13.99) for the 10, 20 and 40 ml/kg bwt groups respectively. There were significant differences between the 10 and 40 ml/kg bwt (P<0.001), and the 20 and 40 ml/kg bwt (P=0.031) treatment groups.

Overall MA values differed significantly by tetrastarch dose (P<0.001) and the mean (s.d.) change from baseline to 12 h was -0.92 (2.92), -2.07 (2.09), and -5.75 (2.09) mm for the 10, 20 and 40 ml/kg bwt groups respectively. The 10 and 40 ml/kg bwt treatment groups were significantly different (P<0.001). Compared to baseline, infusion of 20 ml/kg bwt tetrastarch (130/0.4) caused a significant decrease in MA at 1 (P=0.045) and 24 h (P=0.036) post-infusion, while infusion of 40 ml/kg bwt caused a significant decrease at 0 (P<0.001), 1 (P=0.022), 6 (P=0.006), 24 (P<0.001) and 48 h (P=0.013) post-infusion.

2.6.2 Serum colloid osmotic pressure

In all treatment groups, administration of tetrastarch induced a significant increase in COP values (Table 2.3) and the median (IQR) change from baseline to 12 h was 3.1 (3.0 to 3.2), 1.6 (0.2 to 2.6), and 5.4 (4.8 to 7.8) mmHg for the 10, 20 and 40 ml/kg bwt groups respectively. Significant changes persisted throughout the 24 h post-infusion period for the 10 ml/kg bwt group, throughout the 6 h period for the 20 ml/kg bwt group and to 48 h for the 40 ml/kg bwt group.

2.6.3 Serum creatinine and bile acids concentrations

Infusion of tetrastarch caused a significant decrease in serum creatinine concentration between the 10 and 40 ml/kg bwt treatment groups ($P<0.001$) and the change from baseline to 12 h was -5.17 (2.93), -5.83 (8.13) and -4.83 (3.55) µmol/l for the 10, 20 and 40 ml/kg bwt groups respectively. A significant decrease in creatinine concentration was detected at 0 ($P=0.001$) and at 96 h ($P=0.013$) following infusion of the 20 ml/kg bwt dose, and at 0 ($P<0.001$), 6 ($P=0.005$) and 96 h ($P<0.001$) after infusion of the 40 ml/kg bwt dose (Table 2.3). No significant changes over time were observed for the 10 ml/kg bwt dose. Significant changes were not observed for SBA concentrations in any of the treatment groups.

2.6.4 Packed cell volume, plasma total solids and platelet count

There was a significant effect of dose on PCV (P<0.001), TS (P<0.001) and platelet count (P<0.001). The change in PCV from baseline to 12 h was -1.17 (1.33), -3.83 (2.56), and -3.83 (3.06) % for the 10, 20 and 40 ml/kg bwt groups respectively. The corresponding changes for TS and platelet count were 2.33 (2.34), -3.00 (2.10), -2.00 (1.27) g/l, and -4.83 (10.61), -13.83 (9.54), and -5.33 (18.31) x 10^9/l respectively. All three variables were significantly different between the 10 and 40 ml/kg bwt treatment groups (P≤0.001). Significant decreases in TS and PCV were apparent in the 10 ml/kg group immediately after and up to 1 hour after infusion (Table 2.4). Administration of 20 and 40 ml/kg bwt tetrastarch induced significant decreases in TS and PCV with significant differences persisting up to 6 h after infusion for the 20 ml/kg bwt and up to 12 h for the 40 ml/kg bwt dose. Significant changes were not observed for platelet count in the 10 ml/kg bwt treatment group, but significant changes were detected up to 6 h after infusion of 20 ml/kg bwt and up to 1 h following infusion of 40 ml/kg bwt tetrastarch (Table 2.4).

2.6.5 Venous blood gas analysis

Venous blood gas variables are summarised in Tables 2.5 and 2.6 respectively. Overall, pH was not different among treatment groups (P=0.997). Compared to baseline, infusion of 10 and 20 ml/kg bwt tetrastarch (130/0.4) caused a significant decrease in pH immediately after infusion, while infusion of 40 ml/kg bwt caused a significant decrease at 0 and 1 h after infusion. Venous bicarbonate was significantly lower at 0 and 12 h after infusion of 10 ml/kg bwt. No changes over time were observed in the 20 and 40 ml/kg bwt groups.

Overall, sodium (P=0.219), potassium (P=0.665) and ionised calcium concentrations (P=0.807) were not different among treatment groups. Compared to baseline, infusion of 10ml/kg bwt resulted in a significant increase in sodium at 0 and 6 h after infusion, whilst the administration of 40ml/kg bwt resulted in a significant increase at 0, 1, and 6 h post-infusion. No significant changes were observed for the 20 ml/kg bwt group. No significant changes over time were observed for ionised calcium in after the 10 and 40 ml/kg bwt groups, but a significant increase was observed at 6 h post-infusion in the 20 ml/kg bwt group. No significant changes over time were observed for venous potassium concentration in any of the treatment groups (Table 2.6).

2.7 DISCUSSION

2.7.1 Thromboelastography

In the present study three treatment protocols were evaluated to determine the effect of tetrastarch (130/0.4) on coagulation variables in healthy pony mares. All TEG variables measured remained within established reference ranges. However, the administration of higher doses of tetrastarch (particularly 40 ml/kg bwt) was more likely to induce changes in coagulation as evidenced by a longer K-time and decreased MA compared to after the lower doses. The change in TEG variables over time is consistent with reports describing haemostatic alterations after HES administration in other species [1,12,13]. Hypocoagulation as measured by TEG has also been described in humans receiving HES [17].

The clot strength, represented by MA, is dependent on platelet function, platelet numbers and platelet-fibrin interaction [18]. The observed decrease in MA in the present study could be partially explained by the concurrent observed decrease in platelet count. Despite these reported alterations in haemostatic variables, studies in humans confirm that tetrastarches have minimal effect on coagulation compared to earlier HES preparations [19]. Compared to HES 600/0.75, *in vitro* dilutional effects of HES 130/0.4 did not cause a decreased MA as measured by TEG [15].

In this study, only 2 (MA and *K*) out of 4 variables associated with hypocoagulation differed significantly from baseline within the highest dose (40 ml/kg bwt), whilst only MA differed significantly following administration of the 20 ml/kg bwt dose. Although not measured in this study, other haemostatic alterations associated with HES administration have been reported in horses [1] and llamas [13]. These alterations include decreases in platelet count and von Willebrand factor antigen (vWF:Ag) activity, increased and decreased prothrombin time (PT) and activated prothrombin time (aPTT), as well as decreases in factor VIII coagulant activity.

Thromboelastography was chosen to assess coagulation because it provides information concerning the entire haemostatic system and thus a global assessment of coagulation. Thromboelastography has been evaluated in horses but high variability in measured parameters in healthy horses has also been recognised [20,21]. Only two operators performed TEG in the present study to limit one important source of variability. A limitation involving haemostatic analyses in the present study is that conventional laboratory tests of haemostasis (e.g. PT, aPTT and fibrinogen) were not performed, and haemostatic results can therefore not be directly compared to studies evaluating the effect of HES on these haemostatic variables. This study was also performed in healthy ponies and the effect of HES solutions on coagulation during sepsis and inflammation may differ.

2.7.2 Oncotic effectiveness

Administration of tetrastarch to healthy mares caused a significant increase in COP in all treatment groups, persisting to 24 h and 48 h after 10 and 40 ml/kg bwt respectively. However, COP was only significant to 6 h after tetrastarch infusion at 20 ml/kg bwt. Mean COP values at baseline in horses in the current study were comparable with previously reported values [8,22]. The magnitude of the observed increases in COP were similar to changes previously reported in clinically normal ponies, in which the administration of hetastarch at 10 and 20 ml/kg bwt caused a dose-dependent increase in plasma COP for up to 120 h [1].

Following HES administration, smaller molecules below the renal threshold are rapidly excreted, whereas larger molecules are retained within the vasculature and subsequently hydrolysed into progressively smaller fragments by α-amylase until reaching the renal threshold for excretion [3]. This process causes an increasing number of HES molecules, thereby enhancing the oncotic effect. Thus, a lower MW product will provide more molecules in a given volume of HES solution compared to a high MW product, therefore exerting a greater COP at a similar concentration. The rate of elimination of HES particles is influenced by species-specific differences in plasma α-amylase activity [23,24].

Elimination of tetrastarch in horses has not been reported, however, the prolonged effects on COP observed in this study suggest that there is prolonged intravascular retention of oncotically active particles after infusion. Jones *et al.* [1] reported sustained osmotic effectiveness after hetastarch administration throughout a 120 h observation period. Also, a significant oncotic effect lasting 24 h was reported after

administration of hetastarch to hospitalised horses [8]. Pharmacokinetic differences in HES products based on MW and MS could explain the differences in duration of osmotic effectiveness in this study compared to others.

2.7.3 Haemodilutional effects

Haemodilution is an indirect method of assessing plasma volume expansion and previous studies has shown that erythrocyte mass and total circulating protein is not affected after HES infusion [25]. As expected, infusion of all three tetrastarch doses to healthy horses caused significant haemodilution with dose-dependent decreases in PCV and TS concentration. The observed haemodilutional effect was considerably shorter than the haemodilutional effect reported by Jones *et al.* [1] where total protein values did not return to baseline throughout a 120 h observation period in ponies that received 20 ml/kg HES infusion. Experimental and clinical studies have shown that that the haemodilutional effect of HES are directly related to the dose administered, whereas the duration of effect is dependent on intravascular persistence. The lower MW and MS of tetrastarch (130/0.4) compared to hetastarch (6% HES 450/0.7) used in the study by Jones *et al*. [1] may explain the observed difference in haemodilution.

Third generation HES were developed to enhance degradation and clearance and minimize tissue accumulation. Clearance of HES starch products with a higher MW and MS is much slower compared to products with lower MW and MS [3]. Studies have shown that clearance of tetrastarch is at least 23 times faster than that of hexastarch or hetastarch [3,26]. In humans undergoing orthopaedic surgery, enhanced clearance of tetrastarch was confirmed by 1 mg/ml plasma concentrations of

tetrastarch compared to 2.6 mg/ml for pentastarch by the end of the first postoperative day [27].

An adequate platelet count is essential for normal haemostasis and platelet counts < 100 x 10^9/l can increase cutaneous bleeding time [28]. Hydroxyethyl starch solutions are thought to bind directly to the glycoprotein IIb-IIIa receptor on the platelet surface, preventing outside to inside signalling on the platelet membrane, platelet up-regulation and eventually preventing the formation of a platelet plug and clot [4]. The degree of MS is thought to play a more substantial role in platelet inhibition [29]. A reduction in platelet count has been reported in dogs, humans and horses treated with hetastarch and is attributed to haemodilution due to plasma volume expansion [1,4,12]. Platelet count was not significantly decreased in the 10 ml/kg bwt treatment group in the present study. Although significant reductions in platelet count were observed in the 20 and 40 ml/kg bwt treatment groups, platelet counts did not drop below 100 x 10^9/l and the duration of effect was not as prolonged as that reported by Jones *et al.* [1]. The reduction in platelet count in this study was proportional to the observed decrease in PCV and TS, suggesting that the decrease was associated with haemodilution.

Hydroxyethyl starch administration may influence total solids as measured by refractometer in the present study. To obtain a more accurate representation of serum or plasma protein concentration, chemical analytical methods are preferable.

2.7.4 Effect on renal and liver function

Earlier reports raised concerns about the possible deleterious effects of HES on renal function and recent meta-analyses have confirmed these concerns in critically-ill humans [10,11]. However, evaluation of HES 130/0.4, the preparation used in this study, created no significant difference in 90 day mortality compared to saline when used as a resuscitation fluid for humans in intensive care, though more patients required renal replacement therapy following resuscitation with HES [30]. In contrast, resuscitation with a different formulation HES 130/0.42 (Tetraspan 6%) resulted in both increased mortality and greater need for renal replacement therapy compared to resuscitation with Ringer's acetate [31]. Meta-analyses of randomised, controlled trials of critically ill adult patients involving any formulation of HES concluded that, compared with other resuscitation solutions, the use of HES was associated with a significantly increased risk of mortality and acute kidney injury.

In humans, a high fraction of HES is taken up and deposited in tissues, and renal deposition has been described in humans [32], which may contribute to renal injury. The negative effects reported in humans were not exclusively associated with HES 130/0.4, the preparation used in this study, and were mostly associated with higher dose rates (up to 50 ml/kg/day) than currently recommended for horses. In the present study, which was not designed to investigate renal function in detail, serum creatinine concentration decreased following infusion of tetrastarch in all treatment groups. However, renal function cannot be assessed via measurement of serum creatinine concentration alone, and further investigation with more sensitive methods with a prolonged follow-up period are required to determine possible deleterious effects of tetrastarch (130/0.4) in horses.

Potato-derived HES 130/0.42 is the only tetrastarch reported to be contraindicated in humans with severe hepatic impairment due to induced hyperbilirubinaemia [33]. In the present study serum bile acid concentrations were measured up to 96 hours after-infusion and results were not consistent with impaired liver function. Serum bile acid and creatinine concentrations remained within reference range in 4 out of the 6 mares available for examination 8 months after completion of the study.

2.7.5 Venous blood gas analysis

The acid base and electrolyte changes found in this study were small and considered clinically insignificant. Tetrastarch (130/0.4) is suspended in normal saline (NaCl 0.9%), and therefore contains substantially higher concentrations of sodium and chloride compared to plasma. This could explain the increase in sodium concentration observed in the current study in the 10 and 40 ml/kg treatment groups respectively. A concomitant decrease in pH could be partially explained by the mild but significant decrease in bicarbonate concentration in the 10 ml/kg treatment group. Chloride concentrations were not measured in this study and the anion gap could therefore not be calculated. Hydroxyethyl starch solutions can decrease the anion gap and increase serum chloride concentration, leading to hyperchloraemic acidosis.

2.7.6 Limitations

Other limitations of this study were that the pharmacokinetics of tetrastarch (130/0.4) in horses is unknown and a washout period of 2 weeks was chosen in the absence of specific data concerning the half-life. The rate of elimination of HES particles is influenced by species-specific differences in plasma α-amylase activity [23,24] and amylase activity in equine plasma seems to be lower than amylase activity in humans [24]. In the present study treatments were randomised and adjustments were included in the statistical analyses in an effort to control for any prolonged effects. A further limitation was that data were not recorded between the 1 and 6 h time points and thus the effects of tetrastarch during this period are unknown. Selected time points for blood sampling were chosen based on the expected duration of effect of tetrastarch on the variables under investigation, as well as from previous studies investigating the effect of HES [1]. The limited duration of osmotic effectiveness following infusion of 20 ml/kg bwt tetrastarch could be explained by the limited number of horses used in the study.

Only serum creatinine concentrations were measured in this study and conclusions regarding renal dysfunction cannot be made based on the current results. More sensitive methods and a prolonged follow-up period are needed to evaluate possible deleterious effects on renal function.

This study focused mainly on the effects of tetrastarch on coagulation and platelet function. Assessment of secondary coagulation was not done and additional coagulation tests such as fibrinogen concentration could have provided additional insight. It is well known that TEG results obtained independently from healthy horses

in each laboratory is not interchangeable. As a result, there is a general consensus among researchers that each laboratory should establish their own reference intervals with a standardised TEG technique. The in-house laboratory reference intervals used in this study was established from a very small sample size and is another limitation of this study to consider. However, these reference intervals were established from the same sex and breed as the ponies used in the current study, which could account for less variability.

2.8 CONCLUSION

In conclusion, this study indicates that tetrastarch (130/0.4) administered at 10 and 20 ml/kg bwt has minimal effects on haemostatic variables in ponies as measured by TEG. Although variables remained within reference ranges, compared to lower dosages, infusion of 40 ml/kg bwt tetrastarch is more likely to impact coagulation, as evidenced by an increased coagulation time and decreased overall clot strength. Administration also effectively increased COP, and exerted dose-dependent haemodilutional effects in healthy horses.

Because haemodilutional effects are considered an appropriate indirect means of assessing plasma volume expansion, the observed dose-dependant haemodilutional effects in this study might be related to greater volume expansion at higher doses. Haemodilution might therefore have contributed to the decreases in platelet count and may not be a true reflection of direct *in vivo* effects on coagulation.

The beneficial effects of HES in horses are more likely to be associated with provision of oncotic support during periods of hypoproteinaemia rather than resuscitation as described in humans. Therefore, tetrastarch (130/0.4) has potential as a synthetic colloid in horses. Extrapolation of data between species should be performed with caution, however, and in light of recent concerns in human medicine; further investigation of tetrastarch in critically ill horses is needed to further evaluate potential side effects.

2.9 Figures and tables

Figure 2.1: Tetrastarch 130/0.4, the formulation used in this study.

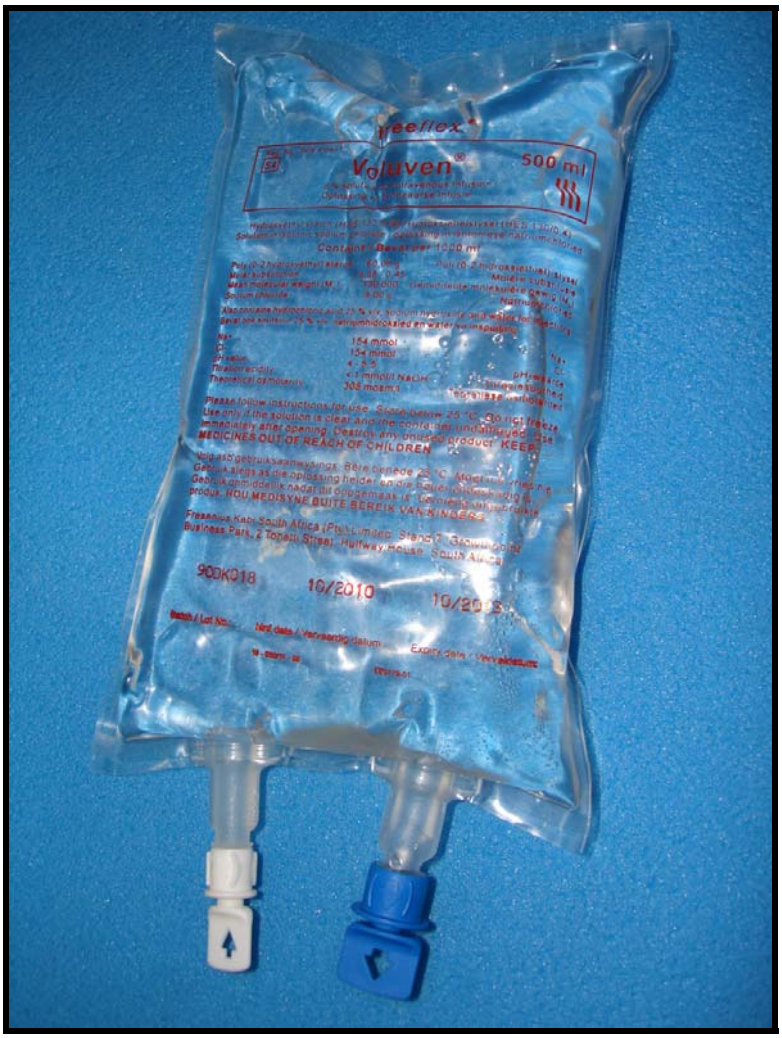


Figure 2.2: Tetrastarch administered to a Nooitgedacht mare with the bags at a pre-determined height.

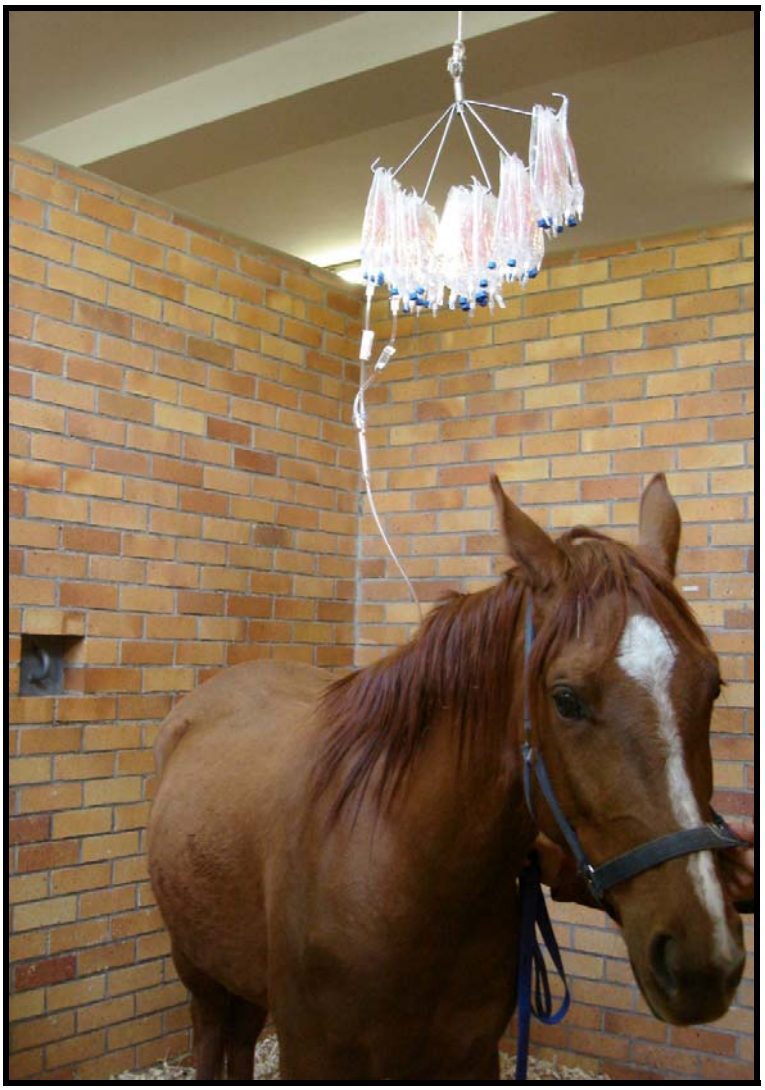
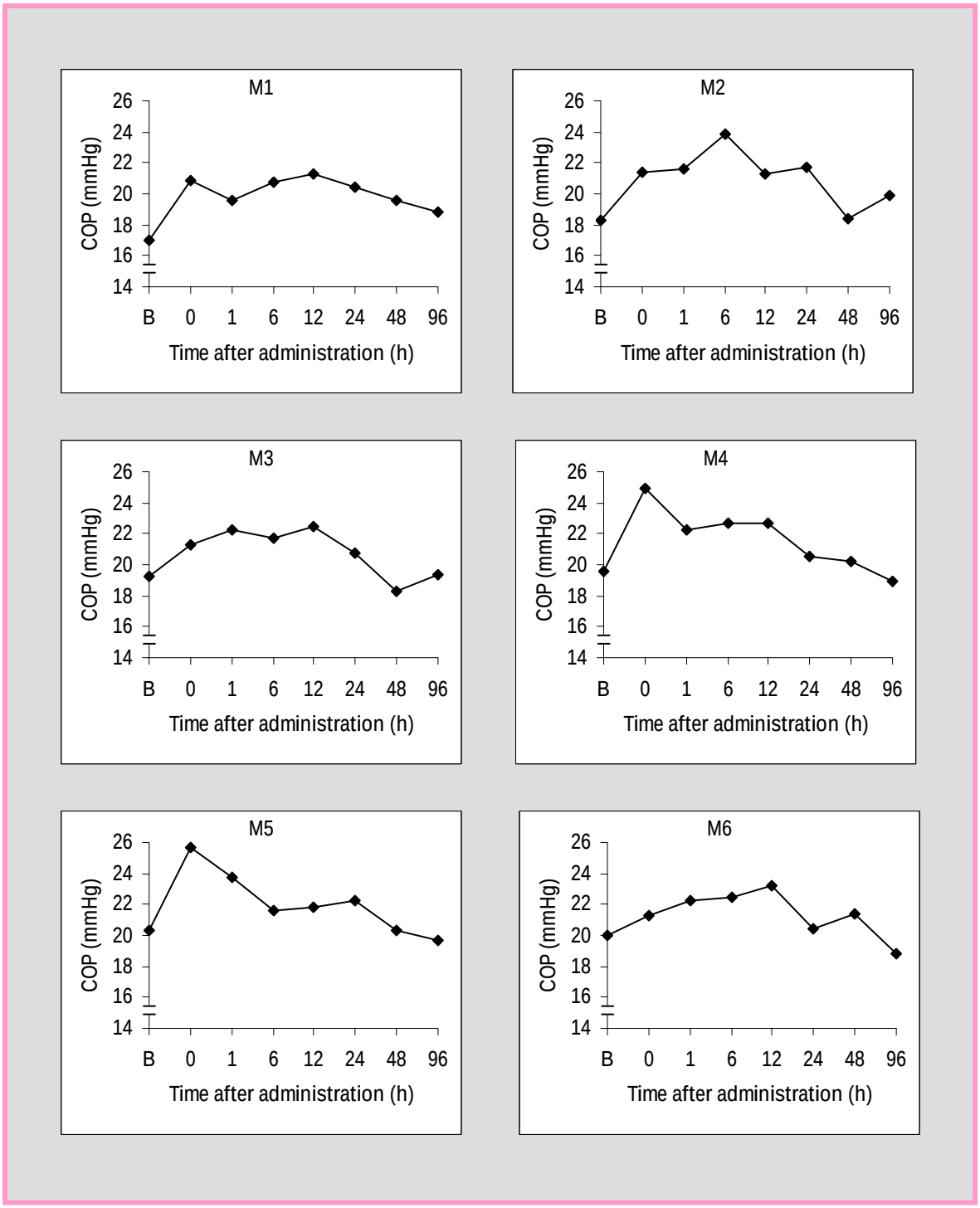
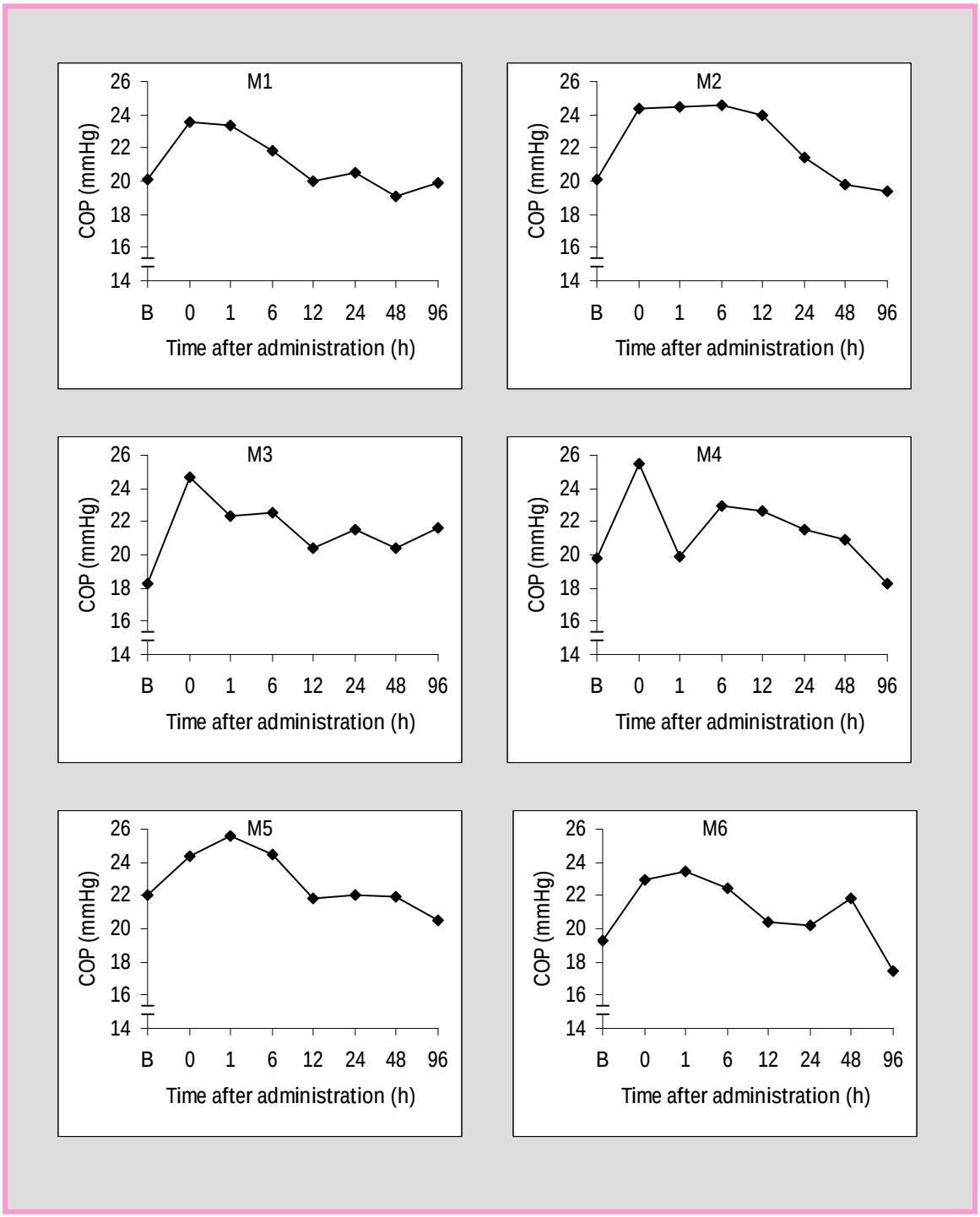


Figure 2.3 Serum colloid osmotic pressure (COP) in 6 healthy mares after i.v. administration of 10 ml/kg bwt tetrastarch (130/0.4).



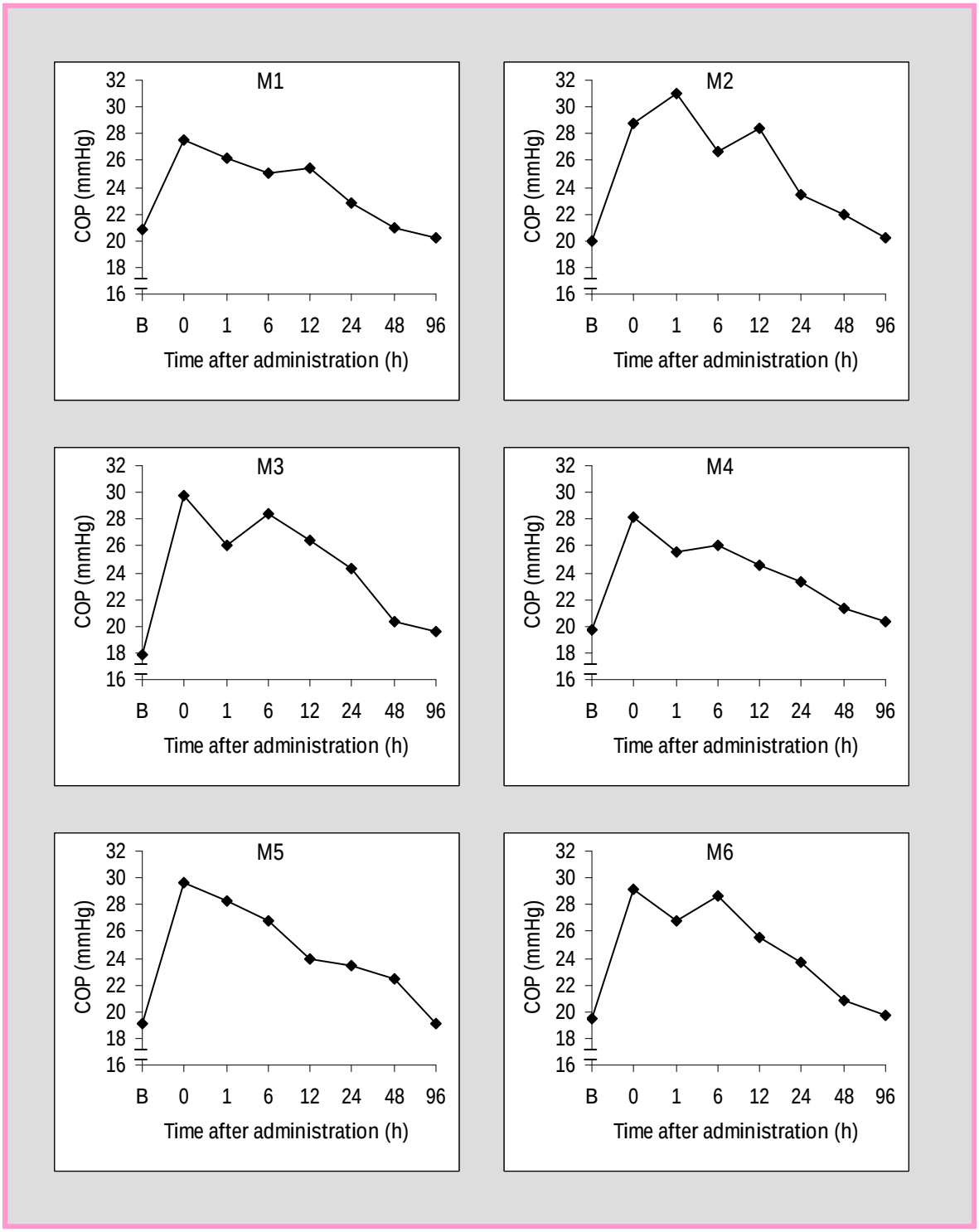
COP: serum colloid osmotic pressure; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.4: Serum colloid osmotic pressure (COP) in 6 healthy mares after i.v. administration of 20ml/kg bwt tetrastarch (130/0.4).



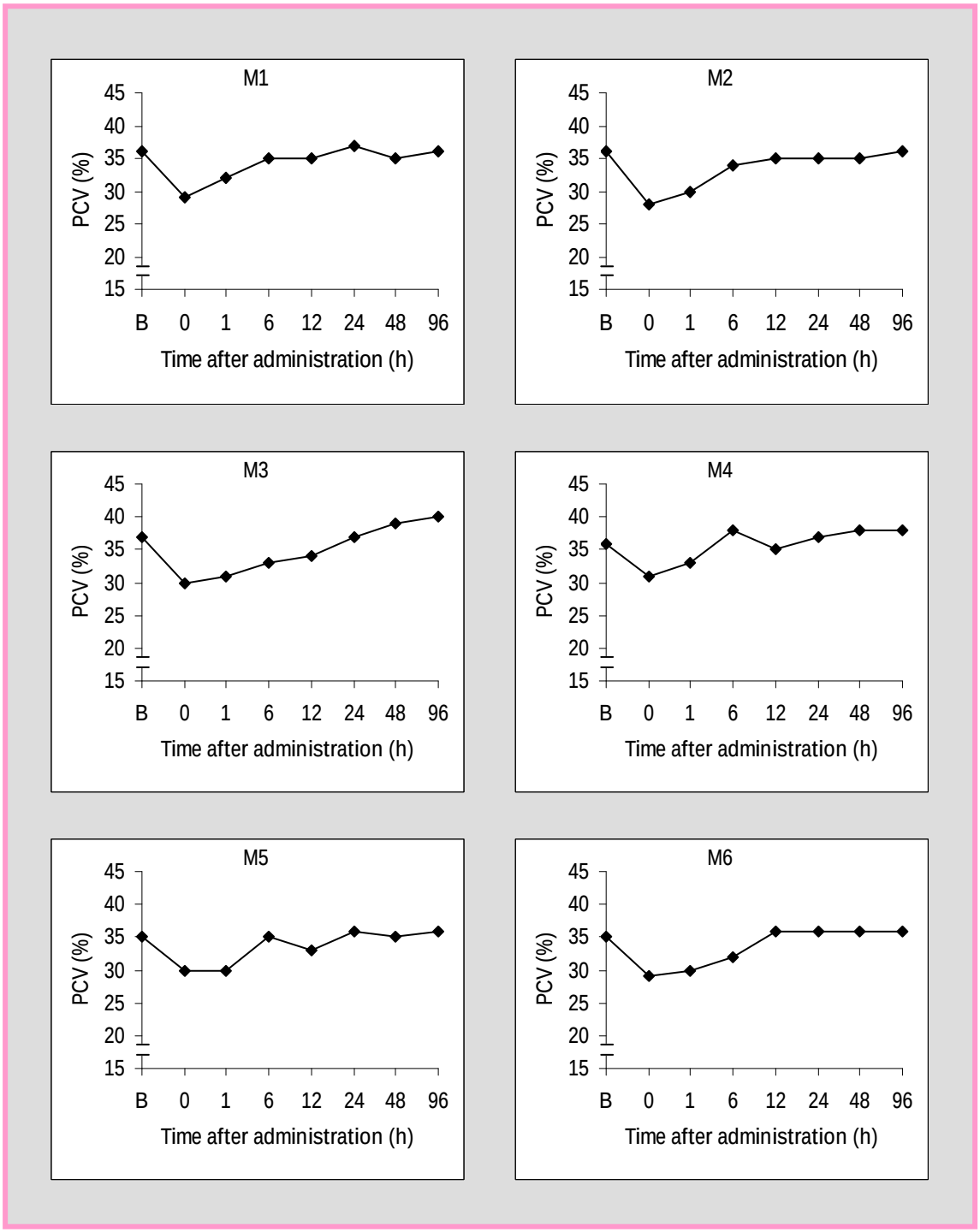
COP: serum colloid osmotic pressure; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.5: Serum colloid osmotic pressure (COP) in 6 healthy mares after i.v. administration of 40ml/kg bwt tetrastarch (130/0.4).



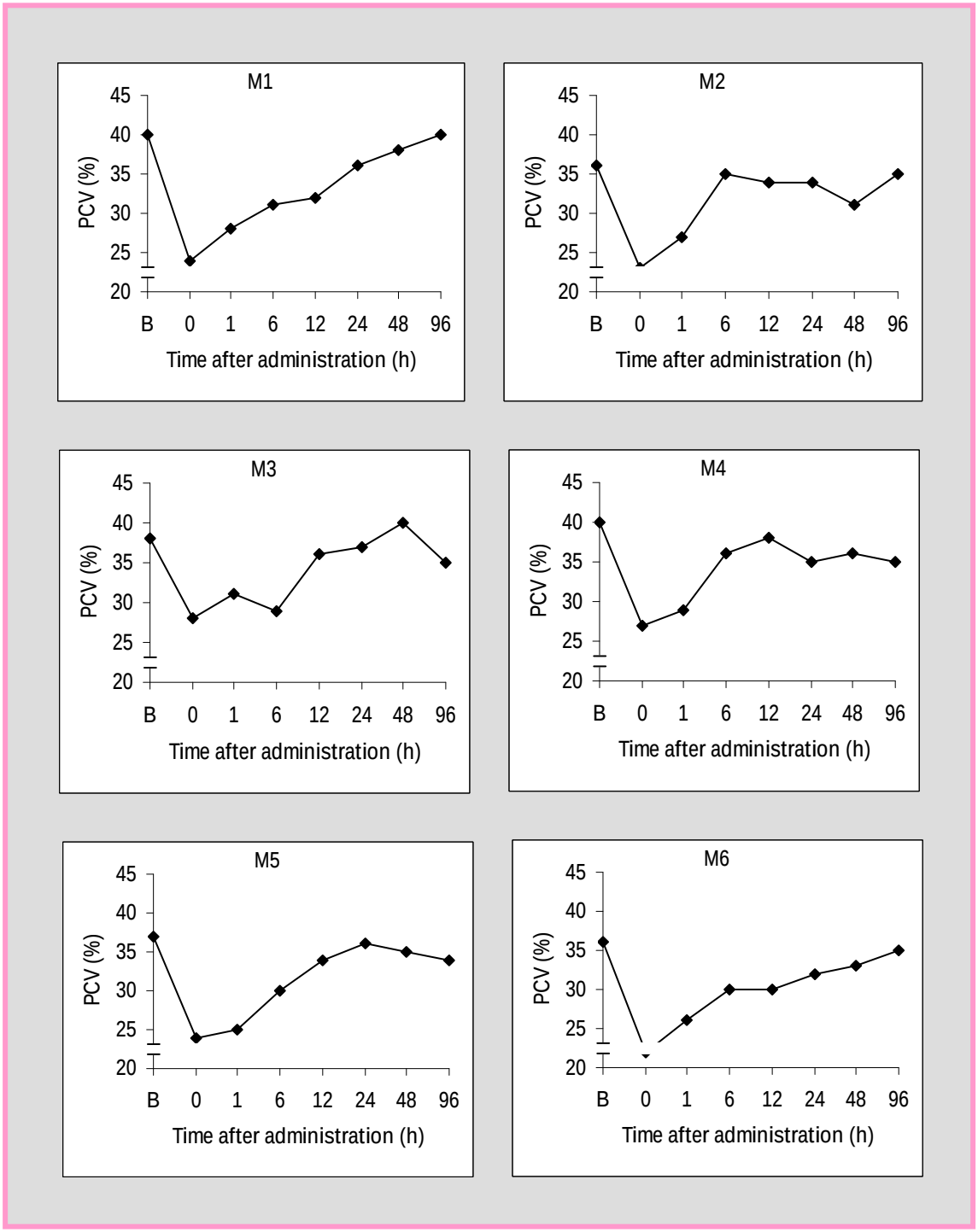
COP: Serum colloid osmotic pressure; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.6: Packed cell volume in 6 healthy mares after i.v. administration of 10ml/kg bwt tetrastarch (130/0.4).



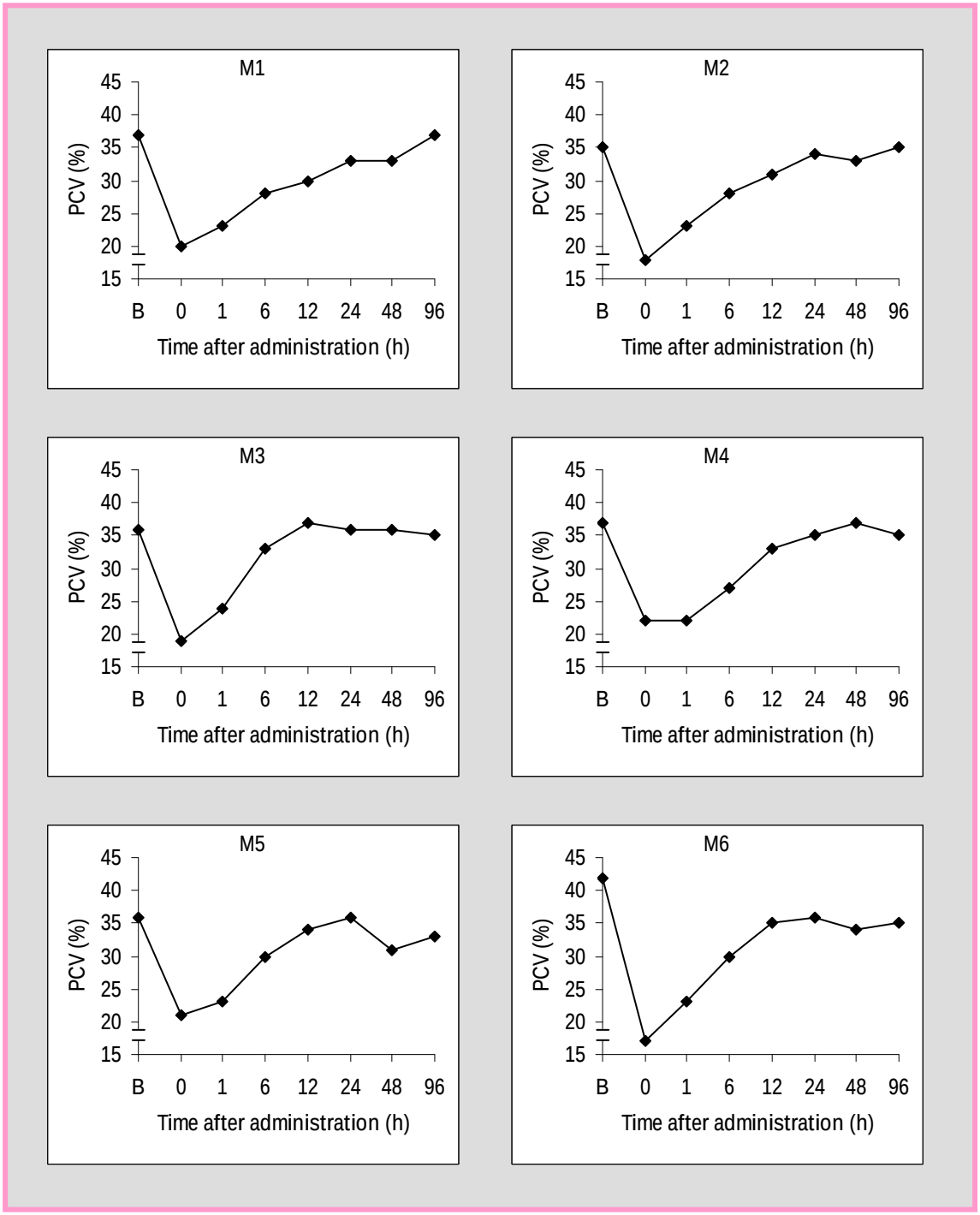
PCV: packed cell volume; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.7: Packed cell volume in 6 healthy mares after i.v. administration of 20ml/kg bwt tetrastarch (130/0.4).



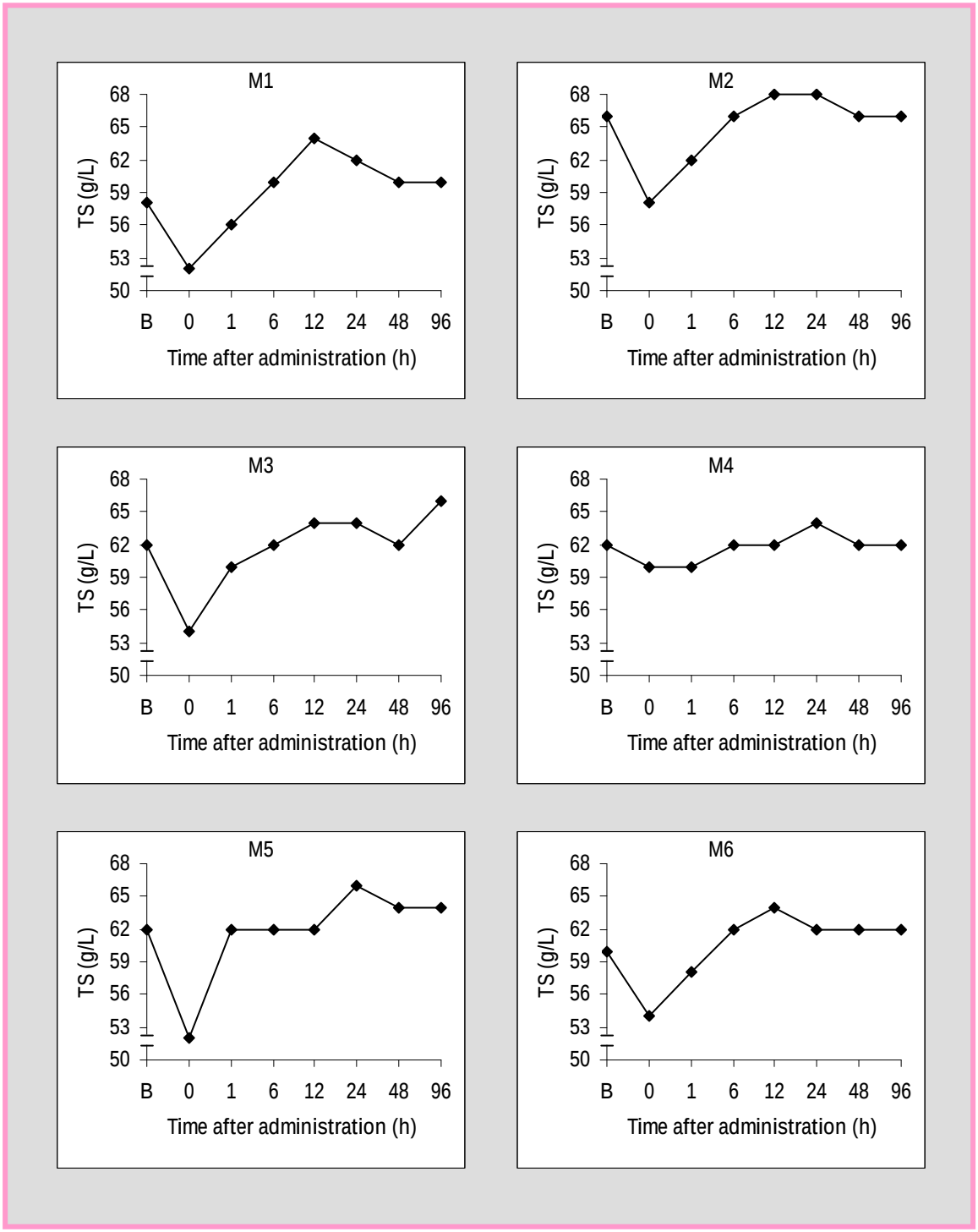
PCV: packed cell volume; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.8: Packed cell volume in 6 healthy mares after i.v. administration of 40ml/kg bwt tetrastarch (130/0.4).



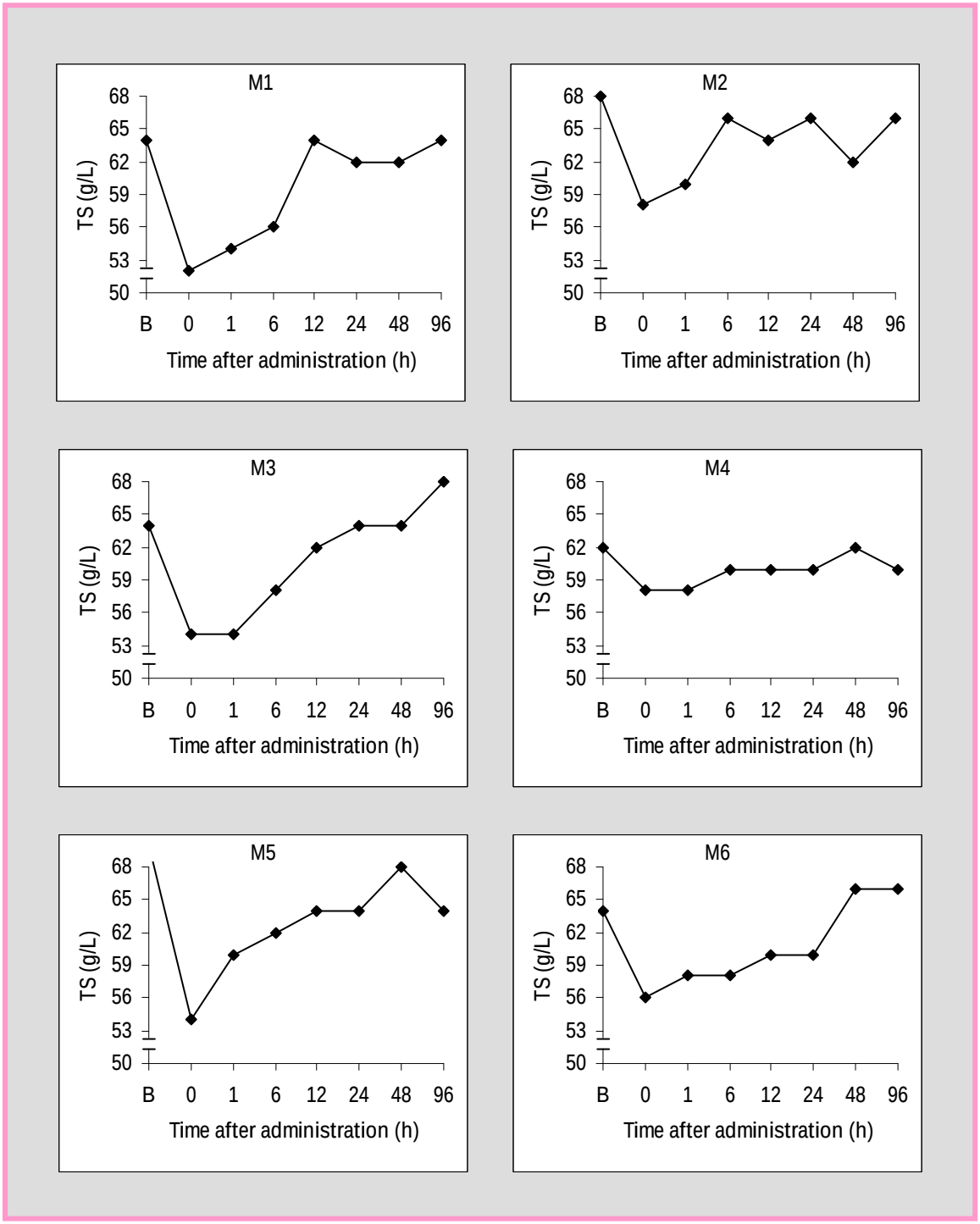
PCV: packed cell volume; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.9: Plasma total solids in 6 healthy mares after i.v. administration of 10ml/kg bwt tetrastarch (130/0.4).



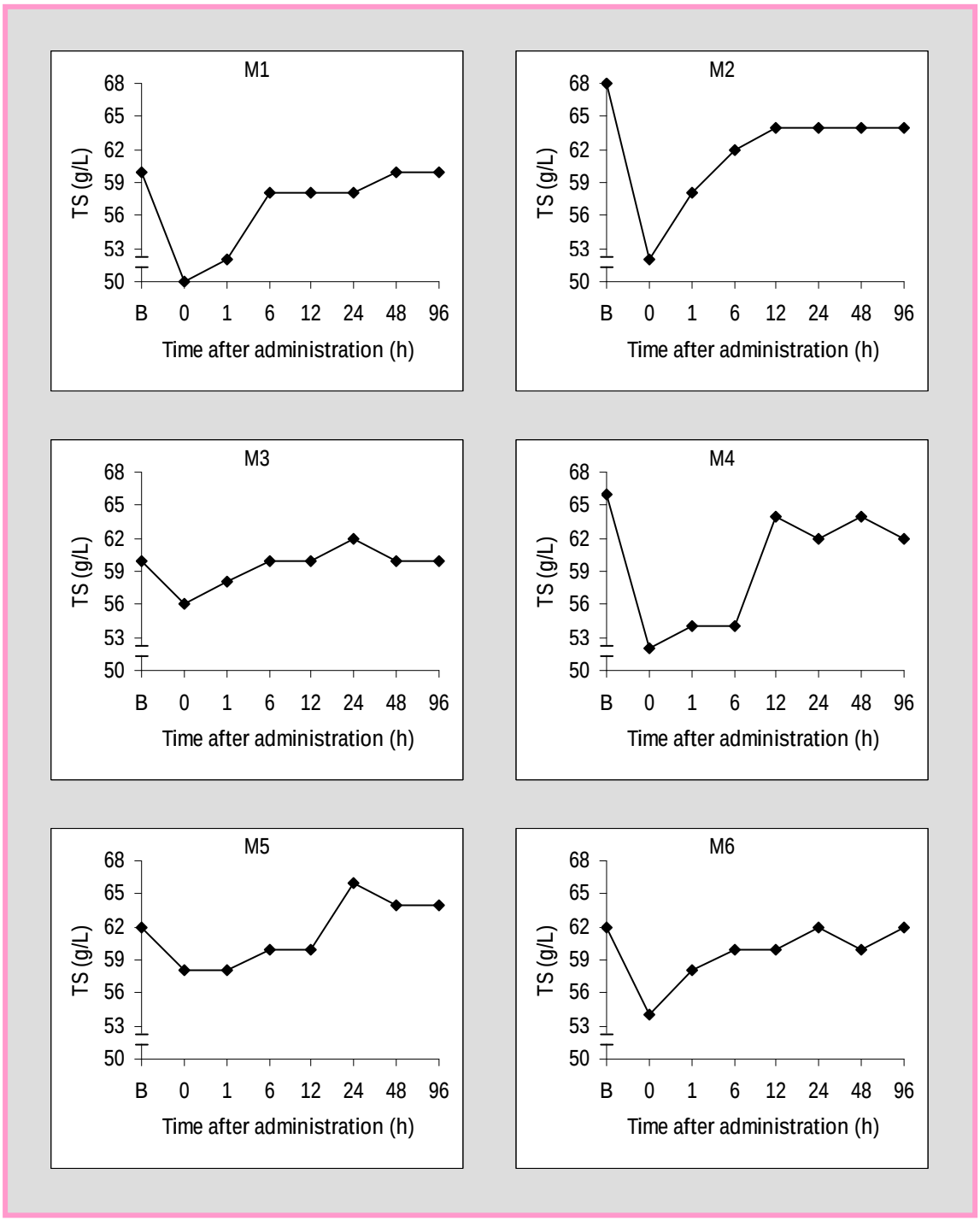
TS: plasma total solids; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.10: Plasma total solids in 6 healthy mares after i.v. administration of 20ml/kg bwt tetrastarch (130/0.4).



TS: plasma total solids; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Figure 2.11: Plasma total solids in 6 healthy mares after i.v. administration of 40ml/kg bwt tetrastarch (130/0.4).



TS: plasma total solids; M1: mare 1; M2: mare 2; M3: mare 3; M4: mare 4; M5: mare 5; M6: mare 6.

Table 2.1: Haemostatic variables (mean ± s.d.) as measured by thromboelastography after i.v. infusion of 10, 20 and 40 ml/kg bwt tetrastarch (130/0.4) in clinically healthy pony mares (n=6). R- and K-time were described as median and interquartile range.

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h	48 h	96 h	†Reference range
R (min)	10 ml/kg	13.1 (12.4-14.8)	13.3 (12.3-15.0)	13.2 (11.6-15.2)	11.8 (11.7-12.7)	16.3 (15.3-16.8)	12.6 (11.9-13.6)	12.5 (11.0-16.2)	9.6 (9.0-10.8)	
	20 ml/kg	17.8 (16.6-19.4)	18.7 (16.7-20.1)	15.1 (14.5-15.5)	14.0 (12.9-14.9)	19.2 (15.3-20.4)	16.3 (15.2-17.6)	15.3 (13.5-16.5)	14.4 (13.1-15.6)	6 - 25
	40 ml/kg	16.9 (14.7-18.0)	17.1 (14.8-21.6)	20.8 (18.1-24.0)	24.6 (22.4-26.0)	21.4 (19.8-25.4)	17.0 (15.6-17.8)	15.0 (12.7-18.1)	10.6 (9.5-14.2)	
K (min)	10 ml/kg	3.4 (2.8-3.7)	3.4 (3.1-3.4)	2.9 (2.5-3.3)	3.0 (2.8-3.0)	3.7 (3.1-3.7)	2.8 (2.4-3.2)	2.8 (2.4-4.3)	2.4 (2.2-2.8)	
	20 ml/kg	4.2 (3.6-4.4)	4.2 (3.6-4.3)	3.2 (3.1-3.2)	3.0 (2.8-3.2)	3.9 (3.4-4.0)	3.6 (3.2-4.0)	3.3 (3.1-3.6)	3.4 (3.2-4.1)	0 - 8
	40 ml/kg	4.3 (3.6-4.6)	4.3 (4.0-8.0)	4.1 (3.8-5.0)	5.5 (5.0-6.4)*	4.7 (4.5-6.3)	3.9 (3.6-4.2)	3.4 (3.0-3.9)	2.7 (2.4-3.1)	
α (°)	10 ml/kg	51.2 ± 5.44	47.9 ± 3.81	51.0 ± 6.19	50.7 ± 5.03	48.1 ± 3.72	51.1 ± 8.62	50.3 ± 8.90	52.7 ± 10.48	
	20 ml/kg	43.8 ± 5.16	45.0 ± 4.74	47.9 ± 4.23	50.4 ± 3.17	41.9 ± 11.28	45.6 ± 6.22	49.4 ± 6.79	44.3 ± 9.38	21 - 68
	40 ml/kg	42.6 ± 7.71	35.3 ± 8.15	39.4 ± 4.72	30.5 ± 11.42	31.5 ± 10.12	40.1 ± 11.06	45.7 ± 6.54	53.0 ± 5.32	
MA (mm)	10 ml/kg	59.3 ± 4.67	57.6 ± 5.0	57.1 ± 3.37	57.8 ± 4.75	58.4 ± 5.13	58.2 ± 3.42	58.5 ± 4.25	57.4 ± 2.84	
	20 ml/kg	63.3 ± 4.13	58.9 ± 6.39	58.4 ± 4.41*	61.3 ± 3.31	61.2 ± 5.73	58.3 ± 5.89*	60.0 ± 5.01	61.3 ± 4.74	50 - 71
	40 ml/kg	62.1 ± 5.97	49.6 ±11.55*	54.8 ± 6.89*	53.7 ± 7.14*	56.3 ± 5.98	51.4 ± 6.57*	54.4 ± 3.57*	58.4 ± 5.53	

*P< 0.05, compared with baseline values; † Internal reference range of 12 healthy pony mares.

Table 2.2: Haemostatic variables (mean ± s.d.) as measured by thromboelastography after i.v infusion of 10, 20 and 40 ml/kg bwt tetrastarch (130/0.4) in clinically healthy pony mares (n=6). G and LY30 were described as median and interquartile range.

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h	48 h	96 h	†Reference range
G (Kd/sc)	10 ml/kg	7.50 (6.75-8.48)	7.0 (6.08-8.0)	6.50 (6.25-6.75)	6.75 (6.10-7.63)	6.80 (6.48-7.65)	7.35 (6.48-7.70)	7.45 (6.25-7.98)	6.85 (6.50-7.28)	
	20 ml/kg	8.55 (7.53-10.03)	7.15 (7.0-8.43)	7.25 (6.35-8.15)*	8.30 (7.50-8.58)	8.05 (6.55-9.40)	6.65 (6.0-7.15)*	7.80 (6.85-8.68)	7.95 (6.90-9.38)	3.8 – 11.9
	40 ml/kg	8.15 (7.45-8.85)	5.25 (4.65-6.83)*	5.65 (5.25-6.20)*	6.05 (4.68-6.90)*	6.40 (5.58-7.75)	5.40 (4.40-6.25)*	5.90 (5.65-6.23)*	6.60 (6.0-8.10)	
LY30 (%)	10 ml/kg	0.35 (0.05-0.88)	0.70 (0.28-1.28)	1.0 (0.48-1.38)	0.60 (0.18-1.10)	1.05 (0.28-1.30)	0.15 (0.0-0.45)	0.30 (0.05-0.78)	0.0 (0.0-0.23)	
	20 ml/kg	0.45 (0.23-0.68)	0.70 (0.33-1.15)	1.25 (0.55-1.58)	1.15 (0.25-1.53)	1.20 (0.93-1.48)	1.05 (0.40-1.18)	0.65 (0.23-0.70)	0.1 (0.03-0.48)	0 - 1
	40 ml/kg	0.40 (0.08-0.50)	0.60 (0.13-1.38)	1.65 (0.85-1.70)	1.50 (0.38-2.10)	1.35 (0.55-1.48)	0.90 (0.63-1.10)	0.35 (0.13-0.65)	0.45 (0.08-0.83)	
LY60 (%)	10 ml/kg	3.75 ± 2.04	4.90 ± 2.15	5.73 ± 2.05*	4.53 ± 1.79	5.06 ± 2.55	3.65 ± 1.82	3.60 ± 1.57	3.02 ± 1.94	
	20 ml/kg	3.92 ± 1.42	5.17 ± 1.58	5.70 ± 2.30*	5.35 ± 2.69	5.68 ± 1.84*	5.30 ± 1.84	4.28 ± 1.26	3.47 ± 1.93	0 - 7
	40 ml/kg	3.47 ± 1.11	4.53 ± 2.93	6.73 ± 2.11*	6.53 ± 4.35*	6.37 ± 1.90*	5.92 ± 2.32	4.02 ± 1.26	4.10 ± 1.79	

*P< 0.05, compared with baseline values; † Internal reference range of 12 healthy pony mares

Table 2.3: Mean ± s.d. serum creatinine (Cr) and bile acids (BA) concentration after i.v. infusion of 10, 20 and 40 ml/kg bwt of tetrastarch (130/0.4) in clinically healthy pony mares (n=6). Serum colloid osmotic pressure (COP) was described as median and interquartile range.

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h	48 h	96 h
COP (mmHg)	10 ml/kg	19.4 (18.5-19.9)	21.3 (21.3-24.0)*	22.3 (21.7-22.3)*	22.1 (21.6-22.6)*	22.1 (21.4-22.6)*	20.6 (20.4-21.4)*	19.9 (18.7-20.2)	19.1 (18.8-19.6)
	20 ml/kg	19.9 (19.4-20.1)	24.4 (23.8-24.6)*	23.4 (22.5-24.2)*	22.7 (22.4-24.1)*	21.1 (20.4-22.4)	21.4 (20.7-21.5)	20.6 (19.9-21.5)	19.6 (18.5-20.3)
	40 ml/kg	19.6 (19.2-19.9)	29.0 (28.3-29.5)*	26.5 (26.0-27.9)*	26.7 (26.2-28.0)*	25.4 (24.8-26.1)*	23.5 (23.2-23.6)*	21.1 (20.8-21.8)*	19.9 (19.6-20.2)
Cr (umol/l)	10 ml/kg	102.8 ± 7.88	94.6 ± 6.28	---	96.1 ± 7.25	97.6 ± 9.41	99.0 ± 9.91	97.6 ± 5.92	113.3 ± 28.55
	20 ml/kg	106.6 ± 16.71	97.0 ± 16.88*	---	101.6 ± 14.36	100.8 ± 12.54	98.6 ± 12.01	100.5 ± 12.19	96.3 ± 12.37*
	40 ml/kg	97.6 ± 11.65	84.0 ± 11.50*	---	89.0 ± 11.13*	92.8 ± 12.43	91.6 ± 10.17	94.0 ± 11.06	83.8 ± 9.10*
BA (umol/l)	10 ml/kg	4.2 ± 1.61	4.0 ± 1.74	---	---	---	6.4 ± 1.54	---	5.8 ± 0.78
	20 ml/kg	5.6 ± 0.98	4.2 ± 0.90	---	---	---	6.8 ± 2.22	---	5.2 ± 1.47
	40 ml/kg	4.6 ± 1.35	7.4 ± 2.02	---	---	---	6.5 ± 1.33	---	5.0 ± 1.41

*P<0 .05, compared with baseline values; ---: Not determined

Table 2.4: Median and interquartile range plasma total solids (TS), packed cell volume (PCV) and platelet count (PC) after i.v. infusion of 10, 20 and 40 ml/kg bwt of tetrastarch (130/0.4) in clinically healthy pony mares (n=6).

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h	48 h	96 h
PCV (%)	10 ml/kg	35.8 ± 0.75	29.5 ± 1.04*	31.0 ± 1.26*	34.5 ± 2.07	34.6 ± 1.03	36.3 ± 0.81	36.3 ± 1.75	37.0 ± 1.67
	20 ml/kg	37.8 ± 1.83	24.6 ± 2.33*	27.7 ± 2.16*	31.8 ± 2.92*	34.0 ± 2.82	35.0 ± 1.78	35.5 ± 3.27	35.6 ± 2.16
	40 ml/kg	37.2 ± 2.48	19.5 ± 1.87*	23.0 ± 0.63*	29.3 ± 2.16*	33.3 ± 2.58*	35.0 ± 1.26	34.0 ± 2.19*	35.0 ± 1.26
TS (g/L)	10 ml/kg	61.6 ± 2.65	55.0 ± 3.29*	59.6 ± 2.33*	62.3 ± 1.96	64.0 ± 2.19	64.3 ± 2.33	62.6 ± 2.06	63.3 ± 2.42
	20 ml/kg	65.3 ± 3.01	55.3 ± 2.42*	57.3 ± 2.73*	60.0 ± 3.57*	62.3 ± 1.96	62.6 ± 2.42	64.0 ± 2.52	64.6 ± 2.73
	40 ml/kg	63.0 ± 3.28	53.6 ± 2.94*	56.3 ± 2.65*	59.0 ± 2.75*	61.0 ± 2.49	62.3 ± 2.65	62.0 ± 2.19	62.0 ± 1.78
PC (x 10^9/L)	10 ml/kg	182 ± 24	169 ± 29	171 ± 25	173 ± 28	178 ± 28	185 ± 28	178 ± 29	147 ± 28
	20 ml/kg	190 ± 25	158 ± 18*	161 ± 19*	174 ± 19*	176 ± 19	181 ± 23	180 ± 23	182 ± 23
	40 ml/kg	172 ± 35	116 ± 51*	138 ± 26*	157 ± 34	167 ± 29	163 ± 30	165 ± 42	162 ± 47

*$P<0.05$, compared with baseline values.

Table 2.5: Venous blood gas variables (mean ± s.d.) after i.v. infusion of 10, 20 and 40 ml/kg bwt of tetrastarch (130/0.4) in clinically healthy pony mares (n=6). Bicarbonate concentration (HCO_3^-) was described as median and interquartile range.

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h
pH	10 ml/kg	7.46 ± 0.01	7.44 ± 0.02*	7.44 ± 0.02	7.45 ± 0.02	7.46 ± 0.02	7.44 ± 0.01
	20 ml/kg	7.44 ± 0.01	7.41 ± 0.02*	7.44 ± 0.01	7.45 ± 0.01	7.45 ± 0.02	7.45 ± 0.01
	40 ml/kg	7.45 ± 0.01	7.41 ± 0.01*	7.43 ± 0.01*	7.46 ± 0.02	7.46 ± 0.02	7.46 ± 0.03
HCO_3^- (mmol/L)	10 ml/kg	32.05 (31.63-33.0)	29.0 (28.37-30.08)*	30.35 (29.48-31.38)	30.95 (30.30-31.90)	29.80 (29.50-29.88)*	30.50 (30.33-31.50)
	20 ml/kg	30.15 (28.43-31.35)	28.05 (27.45-29.18)	28.05 (27.28-28.90)	29.15 (28.43-30.40)	29.65 (29.53-29.78)	30.30 (28.98-31.40)
	40 ml/kg	30.05 (29.03-30.55)	27.40 (26.38-30.30)	28.90 (28.13-38.53)	30.50 (28.85-32.0)	30.85 (29.35-31.68)	30.65 (30.10-31.35)
BE (mmol/L)	10 ml/kg	6.97 ± 1.62	4.32 ± 1.06	5.35 ± 1.06	5.97 ± 0.94	5.25 ± 0.77	5.68 ± 0.73
	20 ml/kg	5.0 ± 1.43	2.98 ± 0.45	3.78 ± 1.24	5.37 ± 2.22	4.83 ± 1.54	5.38 ± 1.25
	40 ml/kg	5.28 ± 1.19	3.32 ± 2.89	7.12 ± 5.62	5.97 ± 1.82	5.83 ± 1.78	7.02 ± 4.19

*$P<0.05$, compared with baseline values.

Table 2.6: Venous blood gas variables (mean ± s.d.) after i.v. infusion of 10, 20 and 40 ml/kg bwt of tetrastarch (130/0.4) in clinically healthy pony mares (n=6). Calcium (Ca^{2+}) was described as median and interquartile range.

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h
Na (mmol/L)	10 ml/kg	134.7 ± 0.8	137.7 ± 2.8*	136.3 ± 2.1	137.2 ± 2.1*	136.2 ± 1.7	136.0 ± 1.4
	20 ml/kg	139.0 ± 2.7	138.8 ± 1.8	139.2 ± 2.2	138.0 ± 1.4	137.8 ± 1.8	137.2 ± 2.3
	40 ml/kg	136.3 ± 2.3	138.2 ± 1.8*	138.7 ± 1.6*	138.2 ± 2.0*	136.8 ± 1.2	135.7 ± 1.9
K (mmol/L)	10 ml/kg	3.78 ± 0.28	3.48 ± 0.40	3.56 ± 0.51	3.48 ± 0.20	3.70 ± 0.20	3.10 ± 0.69
	20 ml/kg	3.39 ± 0.40	3.45 ± 0.47	3.47 ± 0.46	3.76 ± 0.37	3.90 ± 0.21	3.42 ± 0.56
	40 ml/kg	3.83 ± 0.20	3.36 ± 0.40	3.45 ± 0.28	3.34 ± 0.49	3.65 ± 0.32	3.55 ± 0.38
Ca^{2+} (mmol/L)	10 ml/kg	1.49 (1.44-1.51)	1.44 (1.37-1.54)	1.54 (1.53-1.61)	1.47 (1.44-1.49)	1.50 (1.48-1.50)	1.54 (1.52-1.55)
	20 ml/kg	1.48 (1.41-1.55)	1.49 (1.48-1.54)	1.52 (1.51-1.54)	1.57 (1.51-1.59)*	1.53 (1.50-1.56)	1.55 (1.52-1.59)
	40 ml/kg	1.49 (1.46-1.53)	1.47 (1.45-1.47)	1.50 (1.44-1.61)	1.50 (1.46-1.54)	1.50 (1.48-1.52)	1.50 (1.45-1.55)

*$P<0.05$, compared with baseline values.

2.10 References

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2.11 Manufacturer's address

[a]Kruuse, Polyurethane IV catheter, Instavet, Johannesburg, RSA

[b]Voluven®, Fresenius Kabi, Midrand, RSA

[c]Brittan Healthcare, 69 Electron Ave, Electron Park, Isando, RSA

[d]Becton, Dickinson and Company, BD, Beliver Industrial Estate, Plymouth, UK

[e]Cobas Integra 400 plus, Roche, Randburg, RSA

[f]Colloid Osmometer Wescor Model 4420, Fourways, RSA

[g]Advia 2120 Hematology System, Siemens, Isando, RSA

[h]Thromboelastograph Hemostasis System 5000, Pro-Gen Diagnostics (Pty) Ltd, Rivonia, RSA

[i]MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA

[j]IBM SPSS Statistics Version 21, International Business Machines Corp., Armonk, New York, USA

Scientific proceedings associated with this dissertation

Viljoen, A., Page, P.C., Fosgate, G.T. and Saulez, M.N. (2012) The effects of hydroxyethyl starch 6% 130/0.4 on thromboelastography in healthy horses. *31st Forum of the American College of Veterinary Internal Medicine, New Orleans, LA, USA.*

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