

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

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**Conflict of Interest**

No author's conflicts of interest have been declared.

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## **Details to be provided on acceptance**

### **Materials and Methods**

#### *Study design*

Line 84

Nooitgedacht

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Faculty of Veterinary Science Research Committee and the University of Pretoria Institutional

Animal Ethics Committee

#### *Thromboelastography*

Line 131

Nooitgedacht

The study was approved by the Faculty of Veterinary Science Research Committee and the University of Pretoria Institutional Animal Ethics Committee.

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## Summary

**Reasons for performing study:** Clinical indications for hydroxyethyl starches (HES) in horses include rapid plasma volume expansion and oncotic support during periods of hypoproteinaemia. Side effects such as coagulopathies associated with HES administration pose limitations to their use in veterinary medicine. In humans, tetrastarch [hydroxyethyl starch (130/0.4)] has demonstrated less profound effects on coagulation compared to 1st and 2nd generation HES.

**Objectives:** To evaluate the haemostatic and oncotic effects of tetrastarch (130/0.4) administered at 10, 20 and 40 ml/kg bwt in healthy horses.

**Study design:** Randomised crossover study design.

**Methods:** Tetrastarch (130/0.4) was administered to 6 healthy pony mares at 10, 20 and 40 ml/kg bwt with a 2-week washout period. Packed cell volume (PCV), total solids (TS), plasma colloid oncotic pressure (pCOP), platelet count and thromboelastography (TEG) was measured at baseline, immediately after infusion (0 h), 1, 6, 12, 24, 48, and 96 h after tetrastarch infusion.

**Results:** All TEG variables remained within normal reference ranges in all 3 treatment groups. Administration of tetrastarch at 40 ml/kg bwt resulted in a prolonged 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 increased mean pCOP values above baseline in all 3 treatment groups, persisting to 24, 6 and 48 h for the 10, 20 and 40 ml/kg bwt dose respectively.

**Conclusion:** Although still within established reference ranges, compared to lower dosages, 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 pCOP at all dosages evaluated in healthy horses.

**Potential relevance:** 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.

## **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 the high plasma colloid oncotic pressure (pCOP) generated through retention of large osmotically active molecules within the vasculature following 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 different mean molecular weight (MW), degree of molar substitution (MS), C<sub>2</sub>/C<sub>6</sub> ratio and concentration [3]. These parameters determine the pharmacokinetics of the product and potential side effects, including coagulopathies, may be more frequent following the administration of first and second 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 a MS of 0.4 to enhance degradation and limit potential side effects [4]. Preparations can be derived from either maize or potato starch.

Colloids are restricted to the intravascular space resulting in more rapid intravascular volume replacement and expansion compared to crystalloids which 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 has been found to maintain pCOP and plasma volume even when there is increased capillary permeability and albumin leakage [9]. Colloid solutions can therefore be used to restore and maintain pCOP in horses affected by these conditions [8].

Despite potential advantages associated with HES administration, the use of artificial colloids is limited by concerns of reported adverse effects including allergic reactions, coagulopathies, substantially higher costs, and recent reports of an increased risk of renal failure and mortality in critically ill human patients compared with crystalloid administration [4,10,11]. Specific coagulation side effects include decreased circulating factor VIII and von Willebrand factor concentrations, impairment of platelet function and decreased fibrin clot stabilisation [4] and have been described in dogs and horses [1,12]. Other potential complications associated with i.v. colloid administration include haemodilution effects causing decreases in total serum protein and albumin [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 human literature. This study was designed to estimate the coagulation and oncotic pressure effects of tetrastarch (130/0.4) administration in healthy horses. We hypothesised that lower dosages would not be associated with adverse effects on coagulation, and that all dosages administered would increase pCOP.

## **Material and methods**

### ***Study design***

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

### ***Treatment administration***

Treatments were randomly allocated to each mare using a prospective cross-over design. A 12-gauge, single lumen polyurethane intravenous catheter<sup>a</sup> was inserted into

the left jugular vein and sutured into position. Catheters were only used for treatment administration purposes and were removed following each infusion. Tetrastarch (130/0.4)<sup>b</sup> was administered intravenously to each mare once every 2 weeks at three dosages: 10, 20, and 40 ml/kg bwt. 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 connected to a high flow (10 drops/ml) administration set<sup>c</sup> prior to colloid administration.

### ***Blood sampling and analyses***

Venipuncture of the right jugular vein was performed for the measurement of pCOP, packed cell volume (PCV), total solids (TS), platelet count and TEG prior to colloid administration (baseline), immediately after each infusion (0 h), and at 1, 6, 12, 24, 48, and 96 h thereafter. Bile acids were measured at baseline and 0, 24, and 96 h. Blood for PCV and TS analyses was collected into evacuated EDTA tubes<sup>d</sup>. Packed cell volume and TS were determined using microhaematocrit tubes and a refractometer. Blood collected into evacuated serum tubes<sup>d</sup> was used to measure bile acids, creatinine and pCOP using an automated serum biochemistry analyser<sup>e</sup> and colloid osmometer<sup>f</sup>, respectively. Blood collected into an evacuated sodium citrate tube<sup>d</sup> was used for TEG and platelet counts. Platelet counts were determined using an automated analyser<sup>g</sup>.

### ***Thromboelastography***

Thromboelastometric analyses were performed using the Thrombelastograph Hemostatic Analyser<sup>h</sup>. 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 min prior to analysis. Twenty  $\mu\text{L}$  of 0.2M calcium chloride was added to a new preheated ( $37^{\circ}\text{C}$ ) 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  $\mu\text{L}$  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 established from 12 healthy Nooitgedacht pony mares: 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 [ $\alpha$  (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 healthy based on clinical examination and haematological parameters. Reference ranges (minimum and maximum) were established by determining the mean  $\pm$  2 s.d. Two operators performed the TEG analyses and to minimise variability, TEG at baseline and measurements in the immediate post-infusion period, including 6 h after infusion, was performed by the same operator, whilst TEG at 12 h after infusion was always performed by a second operator with the exception of the 48 h TEG analyses of two horses for all 3 treatment administrations and the 96 h analyses of two other horses for all 3 treatment administrations. After TEG analysis had been completed, the remaining blood was centrifuged at 4,000g for 8 min within 1 h of collection and plasma was stored at  $-80^{\circ}\text{C}$ .



## 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<sup>i</sup>. Data that were not normally distributed (R-time, K-time and pCOP) were transformed using either the natural logarithm or by ranking prior to statistical analysis and 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<sup>j</sup> and results interpreted at the 5% level of significance ( $P < 0.05$ ).

## Results

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

Thromboelastometric values at baseline were comparable with the established in-house laboratory reference ranges for kaolin-activated TEG (Table 1). Overall R-time values differed significantly by tetrastarch dose ( $P < 0.001$ ), and the median (IQR) R-

**Table 1:** Haemostatic variables (mean  $\pm$  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 (12.3-5.0)	13.2 (11.6-5.2)	11.8 (11.7-2.7)	16.3 (15.3-6.8)	12.6 (11.9-13.6)	12.4 (11.0-6.2)	9.6 (9.0-10.8)	6 - 25
	20 ml/kg	17.8 (16.6-9.4)	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)	
	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 (2.4-4.25)	2.4 (2.2-2.8)	0 - 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)	
	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)	
$\alpha$ (°)	10 ml/kg	51.2 $\pm$ 5.44	47.9 $\pm$ 3.81	51.0 $\pm$ 6.19	50.7 $\pm$ 5.03	48.1 $\pm$ 3.72	51.1 $\pm$ 8.62	50.3 $\pm$ 8.9	52.7 $\pm$ 10.48	21 - 68
	20 ml/kg	43.8 $\pm$ 5.16	45.0 $\pm$ 4.74	47.9 $\pm$ 4.23	50.4 $\pm$ 3.17	41.9 $\pm$ 11.28	45.6 $\pm$ 6.22	49.4 $\pm$ 6.79	44.3 $\pm$ 9.38	
	40 ml/kg	42.6 $\pm$ 7.71	35.3 $\pm$ 8.15	39.4 $\pm$ 4.72	30.5 $\pm$ 11.42	31.5 $\pm$ 10.12	40.05 $\pm$ 11.06	45.7 $\pm$ 6.54	53.0 $\pm$ 5.32	
MA (mm)	10 ml/kg	59.3 $\pm$ 4.67	57.6 $\pm$ 5.0	57.1 $\pm$ 3.37	57.8 $\pm$ 4.75	58.4 $\pm$ 5.13	58.2 $\pm$ 3.42	58.5 $\pm$ 4.25	57.4 $\pm$ 2.84	50 - 71
	20 ml/kg	63.3 $\pm$ 4.13	58.9 $\pm$ 6.39	58.4 $\pm$ 4.41*	61.3 $\pm$ 3.31	61.2 $\pm$ 5.73	58.3 $\pm$ 5.89*	60.0 $\pm$ 5.01	61.3 $\pm$ 4.74	
	40 ml/kg	62.1 $\pm$ 5.97	49.6 $\pm$ 11.55*	54.8 $\pm$ 6.89*	53.7 $\pm$ 7.14*	56.3 $\pm$ 5.98	51.4 $\pm$ 6.57*	54.4 $\pm$ 3.57*	58.4 $\pm$ 5.53	

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

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) min 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 not the 10 and 20 ml/kg bwt treatment groups ( $P=1.0$ ). No significant changes over time were observed for R-time in any of the 3 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 1). In contrast,  $\alpha$ -angle did not change significantly over time in any of the 3 treatment groups, but there was an overall significant difference by tetrastarch dose ( $P<0.001$ ). The mean (s.d.)  $\alpha$ -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.

In all 3 treatment groups, administration of tetrastarch induced a significant increase in pCOP values (Table 2) 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.

Infusion of tetrastarch caused a significant decrease in 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)  $\mu\text{mol/l}$  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). No significant changes over time were observed for the 10 ml/kg bwt dose. Significant changes were not observed for bile acid concentrations in any of the treatment groups.

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)  $\times 10^9/\text{l}$  respectively. All

**Table 2:** Mean  $\pm$  s.d. creatinine (Cr) and bile acid (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). Plasma colloid oncotic pressure (pCOP) is described as median and interquartile range.

Variable	Dose	Baseline	0 h	1 h	6 h	12 h	24 h	48 h	96 h
pCOP (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 (19.62-20.2)
Cr (umol/l)	10 ml/kg	102.8 $\pm$ 7.88	94.6 $\pm$ 6.28	---	96.1 $\pm$ 7.25	97.6 $\pm$ 9.41	99.0 $\pm$ 9.91	97.6 $\pm$ 5.92	113.3 $\pm$ 28.55
	20 ml/kg	106.6 $\pm$ 16.71	97 $\pm$ 16.88*	---	101.6 $\pm$ 14.36	100.8 $\pm$ 12.54	98.6 $\pm$ 12.01	100.5 $\pm$ 12.19	96.3 $\pm$ 12.37*
	40 ml/kg	97.6 $\pm$ 11.65	84.0 $\pm$ 11.50*	---	89.0 $\pm$ 11.13*	92.8 $\pm$ 12.43	91.6 $\pm$ 10.17	94.0 $\pm$ 11.06	83.8 $\pm$ 9.10*
BA	10 ml/kg	4.2 $\pm$ 1.61	4.0 $\pm$ 1.74	---	---	---	6.4 $\pm$ 1.54	---	5.8 $\pm$ 0.78
	20 ml/kg	5.6 $\pm$ 0.98	4.2 $\pm$ 0.90	---	---	---	6.8 $\pm$ 2.22	---	5.2 $\pm$ 1.47
	40 ml/kg	4.6 $\pm$ 1.35	7.4 $\pm$ 2.02	---	---	---	6.5 $\pm$ 1.33	---	5.0 $\pm$ 1.41

\* $P < 0.05$ , compared with baseline values; ---: Not determined

three variables were significantly different between the 10 and 40 ml/kg bwt treatment groups ( $P \leq 0.001$ ). Significant decreases in TS and PCV concentration were apparent in the 10 ml/kg group immediately post- and up to 1 h after infusion (Table 3). 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 3).

## **Discussion**

In the present study 3 treatment protocols were evaluated to determine the effect of tetrastarch (130/0.4) on coagulation in healthy pony mares. All TEG variables measured remained within established reference ranges. However, the administration of higher doses of tetrastarch (40 ml/kg bwt especially) were more likely to induce changes in coagulation as evidenced by a prolonged K-time and decreased MA. The changes 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, concentration 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 concentration. Despite these reported alterations in haemostatic variables, studies in humans confirm that tetrastarches have minimal effect on coagulation compared to

**Table 3:** Mean  $\pm$  s.d 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 $\pm$ 0.75	29.5 $\pm$ 1.04*	31.0 $\pm$ 1.26*	34.5 $\pm$ 2.07	34.6 $\pm$ 1.03	36.3 $\pm$ 0.81	36.3 $\pm$ 1.75	37.0 $\pm$ 1.67
	20 ml/kg	37.8 $\pm$ 1.83	24.6 $\pm$ 2.33*	27.7 $\pm$ 2.16*	31.8 $\pm$ 2.92*	34.0 $\pm$ 2.82	35.0 $\pm$ 1.78	35.5 $\pm$ 3.27	35.6 $\pm$ 2.16
	40 ml/kg	37.16 $\pm$ 2.48	19.5 $\pm$ 1.87*	23.0 $\pm$ 0.63*	29.3 $\pm$ 2.16*	33.3 $\pm$ 2.58*	35.0 $\pm$ 1.26	34.0 $\pm$ 2.19*	35 $\pm$ 1.26
TS (g/L)	10 ml/kg	61.6 $\pm$ 2.65	55.3 $\pm$ 2.42*	59.6 $\pm$ 2.33*	62.3 $\pm$ 1.96	64.0 $\pm$ 2.19	64.3 $\pm$ 2.33	62.6 $\pm$ 2.06	63.3 $\pm$ 3.01
	20 ml/kg	65.3 $\pm$ 3.01	55.3 $\pm$ 2.42*	57.3 $\pm$ 2.73*	60.0 $\pm$ 3.57*	62.3 $\pm$ 1.96	62.6 $\pm$ 2.42	64.0 $\pm$ 2.52	64.6 $\pm$ 2.73
	40 ml/kg	65.0 $\pm$ 3.28	53.6 $\pm$ 2.94*	56.3 $\pm$ 2.65*	59.0 $\pm$ 2.75*	61.0 $\pm$ 2.49	62.3 $\pm$ 2.65	62.0 $\pm$ 2.19	62.0 $\pm$ 1.78
PC ( $\times 10^9/l$ )	10 ml/kg	182 $\pm$ 24	169 $\pm$ 29	171 $\pm$ 25	173 $\pm$ 28	178 $\pm$ 28	185 $\pm$ 28	178 $\pm$ 29	147 $\pm$ 28
	20 ml/kg	190 $\pm$ 25	158 $\pm$ 18*	161 $\pm$ 19*	174 $\pm$ 19*	176 $\pm$ 19	181 $\pm$ 23	180 $\pm$ 23	182 $\pm$ 23
	40 ml/kg	172 $\pm$ 35	116 $\pm$ 51*	138 $\pm$ 26*	157 $\pm$ 34	167 $\pm$ 29	163 $\pm$ 30	165 $\pm$ 42	162 $\pm$ 47

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

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. Decreases in platelet count and von Willebrand factor antigen (vWF:Ag) activity, increases and decreases in prothrombin time (PT) and activated prothrombin time (aPTT) and decreases in factor VIII coagulant activity have also been reported in horses [1] and llamas [13] receiving HES.

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 for use in horses but high variability in measured parameters of healthy horses has also been recognised [20,21]. Only 2 operators performed TEG in the present study to limit 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 similar studies evaluating the effect of HES on these haemostatic variables. The study was also performed in healthy horses and the effect of HES solutions on coagulation during sepsis and inflammation may differ.

Administration of tetrastarch to healthy mares caused a significant increase in pCOP in all 3 treatment groups persisting to 24 h in the 10 ml/kg and to 48 h in the 40 ml/kg bwt groups. In the present study, osmotic effectiveness was only significant to 6 h



after tetrastarch infusion at 20 ml/kg bwt. Although significance was only observed for up to 6 h, mean pCOP values remained above baseline throughout the 96 h observation period. Mean pCOP values at baseline were comparable with previously reported values for horses [8,22]. The magnitude of the observed increases in pCOP were similar to the changes reported in clinically normal ponies, in which the administration of hetastarch at 10 and 20 ml/kg bwt caused a dose-dependent increase in pCOP for up to 120 h [1]. Osmotic effectiveness depends on the number of oncologically active particles retained within the vasculature and their rate of elimination. 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  $\alpha$ -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 pCOP at a similar concentration. The rate of elimination of HES particles is influenced by species-specific differences in plasma  $\alpha$ -amylase activity [23,24]. Elimination of tetrastarch in horses has not been reported, however, the prolonged effects on pCOP observed in this study suggest that there is prolonged intravascular retention of oncologically 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 in 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.

Haemodilution is an indirect method of assessing plasma volume expansion and previous studies have shown that erythrocyte mass and total circulating protein are not affected after HES infusion [25]. As expected, infusion of all three tetrastarch (130/0.4) doses to healthy horses caused a 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 values throughout a 120 h observation period in ponies that received 20 ml/kg HES infusion. Experimental and clinical studies have shown that the haemodilutional effect of HES are directly related to the dose administered, whereas the duration of effect is dependant on intravascular persistence. 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]. 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.

An adequate platelet count is essential for normal haemostasis and platelet concentrations  $< 100 \times 10^9/l$  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 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, these did not decrease platelet concentrations below  $100 \times 10^9/l$  and the duration was not as prolonged as 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 plasma protein concentration, an automated serum biochemical analyser is preferable. There is, however some evidence that the refractive index correlates well with plasma protein concentration [30].

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]. When HES 130/0.4, the preparation used in this study, and saline were evaluated as a resuscitation fluid for humans in intensive care, no significant difference in 90-day mortality was detected between patients, however more patients required renal replacement therapy following resuscitation with HES [31]. In contrast,

resuscitation with a different formulation HES 130/0.42 (Tetraspan 6%), resulted in both an increased mortality and need for renal replacement therapy compared to resuscitation with Ringer's acetate [32]. 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 significant 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 [33] which may contribute to reported 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 HES used as a resuscitative fluid administered at higher dosages (up to 50ml/kg/day) compared to current recommended dosages in horses. In the present study, which was not designed to investigate renal function in detail, creatinine concentration decreased following infusion of tetrastarch in all 3 treatment groups. Renal function cannot be assessed on creatinine concentration alone and further investigation with more sensitive methods is 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 [34]. In the present study bile acid concentration was measured up to 96 h post-infusion and results were not consistent with impaired liver function. Routine bile acid and creatinine measurements were still normal in 4 out of the 6 mares examined 8 months after completion of the study (the other 2 mares were not examined).

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  $\alpha$ -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 effort to control for any carry-over effect. 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 investigated 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.

In conclusion, this study indicates that tetrastarch (130/0.4) administered at 10 and 20 ml/kg bwt has minimal effects on haemostatic variables as measured by TEG. Although still within normal reference ranges, compared to lower dosages, infusion of 40 ml/kg bwt tetrastarch is more likely to induce dose-related changes in indicators of coagulation, as evidenced by an increased coagulation time and decreased overall clot strength. Administration thereof effectively increased pCOP, and exerted dose-dependent haemodilutional effects in healthy horses. 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 done with caution, however, in light of recent concerns in humans, further investigation of administration in critically ill horses is needed to evaluate potential side effects as described in humans.

**Manufacturers Address:**

<sup>a</sup>Kruuse, Polyurethane IV catheter, Instavet, Johannesburg, RSA

<sup>b</sup>Voluven, Fresenius Kabi, Midrand, RSA

<sup>c</sup>Brittan Healthcare, 69 Electron Ave, Electron Park, Isando, RSA

<sup>d</sup>Becton, Dickinson and Company, BD, Beliver Industrial Estate, Plymouth, UK.

<sup>e</sup>Cobas Integra 400 plus, Roche, Randburg, RSA

<sup>f</sup>Colloid Osmometer Wescor Model 4420, Fourways, RSA

<sup>g</sup>Advia 2120 Hematology System, Siemens, Isando, RSA

<sup>h</sup>Thromboelastograph Hemostasis System 5000, Pro-Gen Diagnostics (Pty) Ltd,  
Rivonia, RSA

<sup>i</sup>MINITAB Statistical Software, Release 13.32, Minitab Inc, State College,  
Pennsylvania, USA

<sup>j</sup>IBM SPSS Statistics Version 21, International Business Machines Corp., Armonk,  
New York, USA

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