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Validity of indicators of dehydration in working horses: a longitudinal study of changes in skin tent duration, mucous membrane dryness and drinking behaviour

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Summary

Reasons for performing study: Dehydration is a serious welfare concern in horses working in developing countries. Identification of a valid and practical indicator of dehydration would enable more rapid treatment and prevention.

Objectives: To examine changes in body weight, clinical and blood parameters during rehydration of working horses, identify a 'gold standard' criterion for dehydration and use this to validate a standardised skin tent test, drinking behaviour and mucous membrane dryness as potential field indicators.

Methods: Fifty horses with a positive skin tent test, working in environmental temperatures of 30 to 44°C in Pakistan, were rested and offered water to drink *ad libitum*. Body weight, clinical and blood parameters, mucous membrane dryness, drinking behaviour and skin tent duration at six anatomical locations were measured at 0, 30, 60, 120, 180, 240 and 300 minutes.

Results: Skin tent duration was affected by side of animal ($p=0.008$), anatomical location and coat moisture (both $p<0.001$). Younger animals had shorter skin tents at all time points ($p=0.007$). There was no significant association between plasma osmolality (P_{osm}) or water intake and skin tent duration. Horses with a higher P_{osm} drank significantly more water ($p<0.001$), and had longer ($p<0.001$) and more frequent ($p=0.001$) drinking bouts. Neither P_{osm} nor water intake affected qualitative and semi-quantitative measurements of mucous membrane dryness significantly.

Conclusions and potential relevance:

The standardised skin tent test and measures of mucous membrane dryness investigated in this study were not valid or repeatable indicators of dehydration when compared with P_{osm} as a 'gold standard' criterion. The volume of water consumed and the number and duration of drinking bouts are the most reliable guide to hydration status currently available for adult working horses. Offering palatable water to drink *ad libitum* provides both the diagnosis and the remedy for dehydration in working horses.

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Introduction

Recognition, prevention and rational management of dehydration would constitute a major contribution to the welfare of equids working in developing countries (Pritchard *et al.* 2006). Many horses work for a median 8.1 (inter-quartile range: 4.25 – 12.5) hours per day, pulling carts or carrying loads at moderate speeds in environmental temperatures of up to 44°C (Pritchard 2007a), so could be considered equivalent to non-elite endurance athletes. In endurance horses, dehydration is a contributing factor to reduction in work capacity (Sosa León *et al.* 1995), exhaustion (Carlson *et al.* 1976) and conditions such as heat stroke and synchronous diaphragmatic flutter (Sosa León 1998).

Dehydration in equids has been evaluated by assessment of clinical signs such as pulse rate and quality, heart rate, capillary refill time, mucous membrane dryness and skin turgor (Rose and Hodgson 2000); measurement of blood parameters including packed cell volume (PCV), serum total protein (TP), electrolytes and osmolality (P_{osm}) (Brownlow and Hutchins 1982) and estimation from known fluid losses (Kingston *et al.* 1997). Changes in body weight (BWT) are considered to be a reliable guide to fluid balance during exercise (Carlson 1987), so dehydration is commonly described in terms of percentage loss of BWT. Guidelines for subjective clinical assessment of dehydration vary considerably both between and within current equine veterinary textbooks (Barton and Moore 1999; Robinson 2003; Rose and Hodgson 2000). Butudom *et al.* (2003) noted that sweat losses following exercise in the horse may be isotonic or hypertonic to plasma (potentially leading to isotonic or hypotonic dehydration) and that dehydration results partly from a mismatch between thirst and water deficit. A primary stimulus for thirst is plasma hypertonicity (Johnson 1998); this may be interpreted to suggest that voluntary water intake may not be a useful indicator of hydration status. Pritchard *et al.* (2006) found a positive correlation between P_{osm} and volume of water drunk by working donkeys but not horses.

The skin tent test examines the delay in return of a fold of pinched skin to its normal position (Dorrington 1981). Application of this test on the neck, point of shoulder or eyelid has been used for the clinical assessment of dehydration in sick animals and equine athletes (Harris *et al.* 1995, Rose and Hodgson 2000, Robinson 2003). Rose and Hodgson (2000) specified that the skin over the point of the shoulder provides more reliable results than that over the neck and described the duration of skin tent as proportional to the degree of dehydration (percentage loss of BWT) it represents. Some investigators have questioned the validity and repeatability of this test in sport horses (Harris *et al*. 1995) and in working horses and donkeys (Pritchard *et al.* 2006, 2007b). In particular, the 3-tier graded skin tent test used by Harris *et al*. (1995) was not a reliable indicator of environmental conditions or performance and individual horses showed marked differences in resting results between the left and right sides of the neck. Pritchard *et al.* (2006) found that for working horses and donkeys, an anatomically standardised skin tent test on the neck using a two-tier grading system (normal/ abnormal) could not be validated using PCV, TP and P_{osm} sampled at a single point in time, due to the potentially confounding effects of anaemia, hypoproteinaemia and electrolyte depletion in the sample population.

The aims of this investigation were:

1) to measure longitudinal changes in PCV, TP, P_{osm}, electrolytes, clinical signs and BWT during rehydration by provision of water to drink *ad libitum*, in order to establish a physiological gold standard criterion for dehydration in adult working horses; and

2) to establish the criterion validity of a standardised skin tent test at three anatomical locations on each side of the animal, an assessment of oral mucous membrane dryness using a novel adaptation of the Schirmer test, and thirst as evidenced by drinking behaviour, as potential field indicators of dehydration.

Materials and methods

This study was carried out in Lahore during May/June 2006, under ethical approval from the University of Bristol (Investigation number UB/04/075) and was compliant with Pakistan law regarding ethical use of animals in science. All clinical observations were made at a field clinic run by the working equine welfare charity, the Brooke Hospital for Animals, using a standard test protocol carried out by a single observer (JCP). Data relating to drinking behaviour were collected by a second observer (RE). Preliminary testing of the method was carried out during a 2-week pilot study in July 2005 and for the first 2 days of the current study period.

Animals

The longitudinal study of 50 horses examined each animal at 7 time points over 5 hours, during which drinking water was offered *ad libitum*. Animals recruited to the study were working in high ambient temperatures (30 to 44°C and 17% to 56% relative humidity) in the vicinity of the clinic, transporting people or goods by cart. The selection criteria were: age 2 to 15 years, body condition score (BCS) 2 to 3 on a scale of 1 (very thin) to 5 (very fat), with a positive skin tent test on admission. Horses presented to the clinic for treatment of disease or lameness were not selected.

Preliminary assessment

On admission the following were recorded: body weight (BWT), using an electronic weighbridge (Eziweigh)[1] previously calibrated with known volumes of water; heart rate (HR); BCS; respiratory rate (RR); and rectal temperature (RT) using a digital thermometer. A jugular catheter was placed anterograde under local anaesthesia and the horse was then rested in the shade for at least 15 minutes prior to the first test.

Test protocol

A 20ml jugular venous blood sample was drawn at time point 0 and at 30, 60, 120, 180, 240 and 300 minutes. Ten minutes prior to each time point, the horse was removed from the pen, weighed three times and the average BWT recorded. A clinical examination was carried out, as described for the preliminary assessment. A vertical fold of skin was pinched and released using the standardised method described by Pritchard *et al.* (2006). This was repeated at 10 second intervals 3 times each over the centre of *m. serratus ventralis* (‘injection triangle’), *m. brachiocephalicus* and the point of the shoulder, repeated on each side of the animal (a total of 18 skin tents). Time taken for the released skin to return to its normal contour was measured in 1/100ths of a second using a hand-held stopwatch. Ten seconds before the first skin tent test in each anatomical position, and then immediately after each one (10 seconds prior to the next one), the skin was smoothed once using the back of the hand: (a) to ensure that it had returned to its normal contour ready for the next pinch, and (b) to assess moisture of the hair coat at this location (see Table 1 for definitions) as one of the following: dry (DD), dried sweat (DS), damp sweat (DaS), wet sweat (WS) or wet with water/ rain (WW).

Mucous membrane dryness

Preliminary testing had indicated that neither ocular tear test strips (Schirmer Tear Test[2]), nor phenol red test threads (Zone-Quick[3]) were suitable for assessing gingival mucous membrane tackiness in the horse, due to practical difficulties with retaining them in a standardised position, so a novel method was devised for this study. During each clinical examination, gingival moisture was assessed immediately dorsal to the upper corner incisor, using a 2cm x 2cm square of fast filter paper (Filpap F4/KA2[4]) placed on the mucosa for 10s and alternating sides of the mouth at each time point. Preliminary testing had also shown that in the high ambient temperatures, rapid evaporation from the filter paper precluded the use of advanced weighing techniques to calculate amount of moisture absorbed, so a quantitative assessment of gum moisture was made by delineating the wet area immediately. This was later transferred to graph paper and the area of wet paper was calculated. Qualitative assessment of dryness and adhesion to the mucosa was scored as shown in Table 2.

Drinking behaviour

The horse was returned to the pen and offered water at ambient temperature from a 30L plastic container, standing in a spill tray. The following components of drinking behaviour were observed for the first 10 minutes after returning to the pen: latency to first drink, number of broken (by raising the head above the bucket rim) and unbroken drinking bouts and average length of drinking bout. While the clinical examination was taking place, volume of water drunk (to the nearest 0.5L) since the previous time point was measured. Water was fully replaced each time in order to minimise effects of water temperature change on drinking behaviour. Water spilled during drinking and water evaporated from an identical container placed outside the pen were measured at each time point and these volumes were subtracted from the apparent volume drunk. Water temperature and environmental temperature and relative humidity (Vaisala HM34)[5] were measured at each time point. Food was withheld during the 5 hour observation period.

Blood sample

An aliquot was centrifuged and analysed immediately for PCV (Haematokrit 20)[6]. The remaining sample was divided between EDTA and SST II Plus vacutainers[7]. Serum was separated (EBA-20)[6] on site and all samples were submitted to the national reference laboratory (Aga Khan University Hospital Laboratories, Karachi) for determination of TP (Cobas Mira)[8], sodium (Na^+), potassium (K^+) and chloride (Cl^-) concentrations (Nova 16)[9] and P_{osm} (Advance Osmometer 3D3)[10].

Data analysis

Exploratory analysis showed most residuals to be Normally distributed; some parameters were log_{10}-transformed to achieve this. First, repeatability of the skin tent test was evaluated using a general linear model (GLM) for repeated measures, to examine the effects of anatomical position, side of animal (left/ right), repetition number and coat moisture on skin tent duration. The Tukey *post hoc* test was used to test for differences between means. Based on these results, the most clinically relevant (first) skin tent was included in further repeated measures GLM taking into account anatomical location and side of animal as blocking variables. This examined its relationship to changes in clinical parameters (HR, BCS, mucous membrane tackiness), blood parameters (PCV, TP, P_{osm}, electrolytes), BWT and water intake over the 7 time points, and to identify significant main effects and interactions. Effects of age were also controlled for as blocking factors. Statistical analysis was performed using Minitab[11] (v.15) and the level of statistical significance was set at $p<0.05$ for all analyses.

Results

Animals recruited to the study are summarised in Table 3.

Repeatability of the skin tent test within and between animals

Skin tent duration was affected significantly by age of animal, with older horses having a more prolonged skin tent at all time points than those aged two to five years. Anatomical position, side of animal and coat moisture also had an effect on the duration of skin tenting: the magnitude and direction of these effects are described in Table 4. There was no significant difference in duration between three repetitions of the skin tent test carried out 10 seconds apart at any anatomical location.

Drinking behaviour

Forty-nine of the 50 horses drank water immediately on entering the pen at time 0. Water intake varied significantly over time: horses drank 8.3 (+/- 1.0) L between 0 and 30 minutes, compared with a maximum of 1.3 (+/- 0.2) L between other time points ($F_{(5, 245)} = 48.58$, $p<0.001$). The maximum water intake between 0 and 30 minutes was 28L and the minimum was 0 L. There were no significant effects of environmental heat and humidity or water temperature on water intake.

Blood parameters

Figures 1 and 2 illustrate changes in blood parameters, body weight and water intake over time. Mean PCV and TP values for the group of 50 horses fell between 0 and 30 minutes then rose gradually to initial levels by 300 minutes ($F_{(1, 295)} = 9.37$, $p = 0.002$ and $F_{(1, 295)} = 38.70$, $p < 0.001$, respectively). Four individuals exhibited a temporary 8 to 10% fall in PCV between 30 and 120 minutes. P_{osm} fell significantly from 283 +/- 1.2 mOsm/L to 274 +/- 0.8 mOsm/L between 0 and 30 minutes ($F_{(6, 294)} = 10.69$, $p<0.001$), remaining at this level until 300 minutes. There were no significant relationships between changes in PCV or TP and changes in P_{osm}. Horses with a higher P_{osm} drank significantly more water ($F_{(1,200)} = 945.47$, $p<0.001$), and had longer ($F_{(1,200)} = 15.75$, $p<0.001$) and more frequent ($F_{(1,200)} = 10.64$, $p=0.001$) drinking bouts during each subsequent observation period. However, P_{osm} did not seem to influence the proportion of broken to unbroken drinking bouts. Serum Na^+ and Cl^- followed the same pattern of changes as P_{osm}, while K^+ did not change significantly over the course of the study. There were no significant effects of sex, age or BCS on blood parameters.

Body weight and clinical parameters

Figure 1 illustrates the significant initial increase followed by decrease in BWT ($F_{(6, 294)} = 45.36$, $p<0.001$) recorded over the 5-hour period. The magnitude of BWT change was positively associated with water intake ($F_{(1, 247)} = 945.47$, $p <0.001$) and negatively associated with P_{osm} ($F_{(1, 247)} = 6.24$, $p = 0.013$). Animals with higher heart rates drank larger volumes of water ($F_{(1,129)} = 8.01$, $p = 0.005$). There were no other significant relationships between P_{osm} or water intake and any other clinical parameters measured.

The study found no significant relationship between P_{osm}, PCV or TP and skin tent duration at any anatomical location. There was no significant relationship between P_{osm}, water intake or environmental temperature and humidity and qualitative or quantitative assessments of mucous membrane dryness.

Discussion

Body weight and blood parameters as a ‘gold standard’

Evaluation of skin tent duration and other field measures of dehydration requires a ‘gold standard’ criterion against which to compare their validity (Bland and Altman 1999). In working equids identifying this criterion has been an elusive goal for two reasons: firstly, the confounding effects of sub-clinical disease, excessive sweat electrolyte losses and poor nutrition on standard blood measures such as PCV, TP and P_{osm} (Pritchard *et al.* 2006), and secondly the lack of controlled conditions for accurate measurement of fluid losses. Carlson (1987) recommended measurement of body weight change as an indicator of dehydration. However, Marlin *et al.* (1995) described estimation of fluid losses from measurements of body weight in field situations as being subject to several errors; in particular, the inability to measure faecal and urinary losses. In the current study it was not possible to provide food measured accurately, or calculate fluid and faecal losses, so changes in BWT were not suitable criteria against which to validate other measures. After the predicted rise at 30 minutes, BWT fell by 60 minutes to below its value at time 0 and initial BWT was not regained by 300 minutes. This suggests that the weight of ongoing faecal, urinary and sweat fluid losses over the 5-hour period was greater than that of the water consumed.

Longitudinal measurement of PCV, TP, P_{osm} and electrolytes resulted in a clearer understanding of their relationship with water intake and skin tent duration than was provided by the previous cross-sectional study (Pritchard *et al.* 2006). Figure 2 shows that P_{osm} (with Na^+ and Cl^-) fell by 8 +/- 1.2 mOsm/L after the first drinking bout and maintained a steady state until 300 minutes. This agreed with findings by Butudom *et al.* (2003) who observed P_{osm} and Na^+ returning to normal within 30 minutes of rehydration of horses by offering water to drink. For the current investigation, P_{osm} was selected as the criterion against which to test the validity of other parameters, although as discussed by Bland and Altman (1999), this does not imply that it was a perfect measure. It is notable that only 3 out of 50 values for P_{osm} at time point 0 lay above the reference range established for working horses in Pakistan (272 – 297 mOsm/L; JCP, unpublished data); therefore a single blood sample taken at a this point would not have identified dehydration. However, longitudinal assessment of falling P_{osm} over the course of the study enabled comparison with changes in other variables of interest.

PCV did not demonstrate a relationship with either water intake or P_{osm}. The significant fall in TP seen between 30 and 120 minutes was presumably caused by haemodilution, after which values returned to initial levels. This demonstrates the potential weakness of relying on PCV and TP to assess dehydration: the longitudinal changes in these parameters seen in the present study suggested that animals were euhydrated at time 0. The 8-10% drop in PCV occurring in 4 horses after ingestion of large volumes of water at the initial drinking bout did not alter clinical parameters or appear to lead to abdominal pain. In working horses that may have a low PCV due to concurrent disease, malnutrition or parasitism, there is a theoretical risk of acute anaemia and tissue oxygen deprivation with sudden haemodilution to this extent (Freitag *et al.* 2002).

Repeatability and validity of the standardised skin tent test

The major findings of this study were that the skin tent test, even when standardised in method and timed accurately using a stopwatch, was not repeatable between the right and left sides of the animal or between the three anatomical locations tested. Changes in skin tent duration over time can not be attributed to changes in P_{osm} or to volume of water drunk, as no significant associations between skin tent and measures of hydration status were found in this sample population. These results suggest that changes in skin tent may be attributable to changes in coat moisture, or to other factors such as the apparent effect of small differences in neck position and muscle (including panniculus) movement observed in the preliminary testing periods.

Coat moisture had a highly significant effect on skin tent duration. Investigation of this factor was prompted by an observation, made during the preliminary testing period, that a normal (rapid) skin tent in dry horses became very prolonged (> 15 s) when the animals were suddenly soaked with rain. In the study, the absence of rain or water on the coat meant that no animals were scored WW; however, the order of skin tent duration of DS< DD< DaS< WS suggested that coat moisture may affect skin tent in a graded manner. This could be due to an internal or external effect of sweat production on the elastic recoil of the dermis. A recent review of sweating in horses (Jenkinson *et al.* 2006) found that the myoepithelial cells of the equine subdermis appear contracted during sweating, and described the extrusion of cell vesicles and dead secretory cells, as well as large quantities of electrolytes, from the sweat glands of the dermis. Myoepithelial contraction and either loss of this secretion from dermal sweat glands, or its gain on the skin surface, could potentially affect skin recoil. However, this would not explain the effect of rain water seen in the pilot study.

Skin tents on the left side of the animal were longer than on the right. The assessor's right hand was used to pinch the skin at all three locations on the left side of the horse (and vice versa) so, despite practice, right-handedness may have had an unintentional effect on the strength of the pinch and hence the duration of tenting. Harris *et al.* (2005) found skin tents to be longer on the right side of the neck than the left, but the laterality of the assessor was not described. There may be an additional effect of laterality on reaction times to operate the stopwatch which was held in the opposite hand to that used for pinching. Alternatively, where carts are driven on the left side of the road, as in Pakistan, a difference in muscle size and/or tension on the horse's left side could cause the asymmetry of skin tent duration seen in this study.

Rose and Hodgson (2000) recommended the skin over the point of the shoulder as more reliable than the neck for detection of dehydration; however during rehydration of the horses in the current study, no change in skin tent duration occurred at this location. The finding that skin tent was longest over *m. serratus ventralis*, followed by *m. brachiocephalicus* and shortest, with least variability, over the point of the shoulder may be due to differences in skin tension between these locations. Age-related differences in skin elasticity between animals may explain, at least in part, the significant effect of age on skin tent duration seen in the current investigation and previously (Pritchard *et al.* 2006). Both found that younger horses had shorter skin tent times than older ones, although the age brackets varied slightly between studies.

Validity of mucous membrane dryness and drinking behaviour

The novel quantitative and qualitative assessments of mucous membrane dryness made in the present study were not found to be valid measures of dehydration. Despite subjective assessment of gum dryness or tackiness being recommended in equine clinical publications as part of an assessment of dehydration (Hollis and Corley 2007, Robinson 2003, Rose and Hodgson 2000), evidence from the current study did not support its reliability for this purpose. These findings may be due to other factors over-riding the effect of dehydration; for example, oral mucous membrane dryness could be decreased by drinking or increased by sympathetic stimulation during mouth handling. Alternative sites, such as vaginal and ocular mucous membranes, were investigated during preliminary testing and judged unsuitable for practical reasons, such as the high prevalence of ocular discharge in the working animal population (Pritchard *et al.* 2005).

The pattern of drinking behaviour illustrated in Figure 1 shows that animals appeared to quench their thirst immediately on being offered water and their intake remained low for the rest of the study period. This agrees with Butudom *et al.* (2003) who observed that following a 45-km endurance exercise test, horses drank as soon as water was offered and consumed the majority of their intake within 1 to 2 minutes. Houpt *et al.* (1989) found that resting ponies deprived of water for 24 hours also drank to satiety within 90 seconds of gaining access to water, often in a single long draught, and their fluid deficits were corrected precisely within 15 minutes. In the present study, the number and average duration of drinking bouts reflected P_{osm} but thirst did not reduce the number of times a horse raised its head while drinking, indicating that the need for vigilance or respite during bouts of drinking appears to interrupt the thirst drive for short periods. Therefore, although not ideal, water consumption appears to be the best field test for dehydration. It has the advantages of being simple to administer and simultaneously alleviating the fluid deficit, although there are also potential limitations where water is unavailable, or drinking is inhibited by human proximity (such as an animal in this study that would not drink in the presence of the observer) or negative alliesthesia for water. Although water intake in this study did not appear to be affected by water temperature, voluntary replacement of fluid losses in working horses may be further improved by offering water at optimal temperature, flavour and salinity; this is a potential area for further research.

Conclusions and potential relevance

The dual aims of this study were to define a ‘gold standard’ criterion for identifying dehydration in adult working horses and to validate simple indicators, including a standardised skin tent test, for field use. The gold standard was defined as P_{osm}, subject to the limitation that in dehydrated working horses it frequently may fall within the reference range so longitudinal changes should be examined. Substantial variability in test methodology, anatomical location and position of the animal’s head, neck and limbs when the test is carried out has made skin tent duration subjective and difficult to interpret. The results demonstrated a lack of validity of both a highly standardised skin tent test and measures of gingival mucous membrane dryness when compared to P_{osm} and water intake, with implications for both clinical practice and the assessment of horses during work or competition. Use of drinking behaviour as a field assessment of hydration status constitutes diagnosis by response to treatment. This has disadvantages if drinking is inhibited by internal factors such as negative alliesthesia for water or external factors such as fear of the environment, behaviour of their owners or water availability. However, for working horses, offering palatable water to drink *ad libitum* provides both a simple diagnosis and a remedy for dehydration which can be implemented by any person in the field.

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Manufacturers' addresses

[1]Tru-test Ltd., Auckland, New Zealand.

[2]Schering-Plough Animal Health, New Jersey, USA.

[3]Menicon Pharma, Illkirch-Graffenstaden, France.

[4]Smith Filters, Tunbridge Wells, UK.

[5]Vaisala Group, Vantaa, Finland.

[6]Hettich Zentrifugen, Tuttlingen, Germany.

[7]BD Vacutainer, Pre-analytical Solutions, New Jersey, USA.

[8]Roche Instrument Centre, Rotkreuz, Switzerland.

[9]Nova Biomedical, Massachusetts, USA.

[10]Advance Instruments, Philadelphia, USA.

[11]Minitab Ltd., Coventry, UK.

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Table 1

Qualitative assessment of coat moisture in 50 working horses. [1] 'Feel' assessed by smoothing the hair once with the back of the hand immediately prior to skin tent test

Code	**Name**	**Description of hair coat over anatomical location where skin tent test carried out**	
		Appearance	**Feel**[1]
DD	Dry	Hairs smooth and separated, may be slightly raised.	Dry and smooth
		Colour normal (same as other dry areas of coat).	
DS	Dry sweat	Hairs matted, may be visible pale salt on hairs	Crunchy or crispy texture
		Colour normal.	
DaS	Damp sweat	Hairs smoother than score DD, not separated.	Slightly damp or oily.
		Little or no colour change.	No water transferred to hand.
WS	Wet sweat	Hair and skin visibly soaked.	Wet: slippery or oily texture.
		Distinct colour change (darker).	Water transferred to hand.
WW	Wet water	Hair and skin visibly soaked.	Wet: more 'squeaky' than slippery/ oily texture.
		Distinct colour change (darker).	Water transferred to hand.

Table 2

Qualitative assessment of mucous membrane dryness in 50 working horses, using a 400mm^2 square of filter paper placed on the gingival mucosa, dorsal to the upper lateral incisor, for 10 seconds.

Score	Adhesion	Dryness
0	Falls off mucosa within 10s	Dry
1	Adheres to mucosa	Dry
2	Adheres to mucosa	Wet over 50% of area or less
3	Adheres to mucosa	Wet over greater than 50% but less than 100% of area
4	Adheres to mucosa	Wet over 100% of area
5	Slides off mucosa within 10s	Wet over 100% of area

Table 3

Description of 50 working horses recruited to study with a positive skin tent test

Parameter	Mares (*n* = 43)	Stallions (*n* = 7)
Age		
2 - 5 years	6	1
6 - 10 years	14	1
11 - 15 years	23	5
Body condition score (BCS)		
2	37	7
2.5	5	0
3	1	0

Table 4

Effects of age, anatomical location, side of animal, coat moisture and repetition on skin tent duration in 50 working horses.

[1] n/s = not significant

[2] not an exact F test

Variable	$F_{(df,error)}$	Significance[1]	Description/ direction of effect on skin tent duration
Age of horse	$F_{(2, 2024)} = 5.44$[2]	$p = 0.007$	older age groups > 2 - 5 years
Anatomical location	$F_{(2, 2024)} = 899.61$	$p < 0.001$	*M. serratus ventralis* > *m. brachiocephalicus* > point of shoulder
Side of animal	$F_{(1, 2024)} = 6.98$	$p = 0.008$	Left side > right side at all anatomical locations
Coat moisture	$F_{(3, 2024)} = 37.74$	$p < 0.001$	Wet sweat > damp sweat > dry coat > dried sweat
Repetition	$F_{(2, 6241)} = 1.10$	n/s	No difference between 3 repetitions 10 seconds apart

Figure 1

Changes (mean + s.e.) in osmolality, body weight and water intake during rest and rehydration of 50 working horses

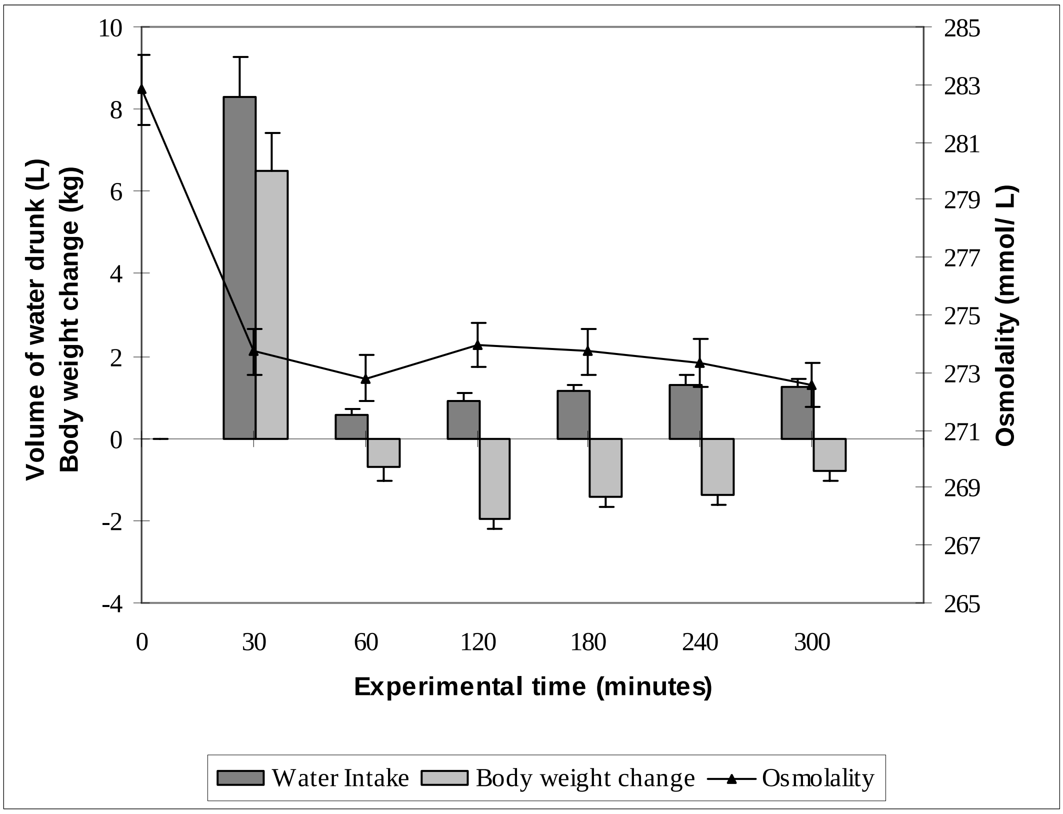
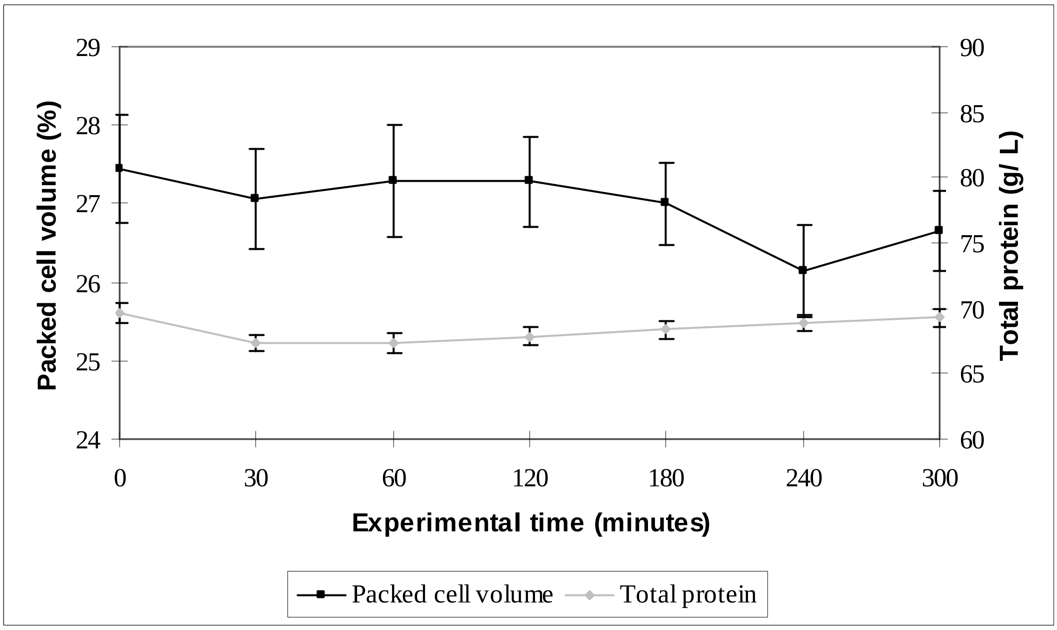


Figure 2

Changes in blood parameters – (A) PCV, TP, and (B) electrolytes – during rest and rehydration of 50 working horses

(A)



(B)