## Title page: Determination of reference intervals for equine arterial blood gas, acid-base and electrolyte analysis

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### Running Head

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### Authors’ contributions

JH: statistical analysis and preparation of manuscript.

DB: concept and design of the study, data acquisition and critical revision of the manuscript.

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**Determination of reference intervals for equine arterial blood gas, acid-base and electrolyte analysis**

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# Abstract (299)

# Objectives To establish reference intervals for arterial blood-gas, acid-base and electrolyte values from a healthy equine population.

# Study-Design Retrospective clinical study

# Animals A total of 139 client owned, systemically healthy horses, 1 year of age and older, presented for elective surgical procedures.

# Methods Blood samples were collected anaerobically from the transverse facial or common carotid artery of horses breathing room air, prior to administration of pre-anaesthetic medication. Samples were analysed immediately, without correction for body temperature, using an automated bench top analyser. Variables analysed included pH, PaCO2 and PaO2, plasma concentration of sodium (Na+), potassium (K+), calcium (Ca2+) and chloride (Cl-). Actual and standardised plasma bicarbonate concentration [HCO3-(P) and HCO3-(P, st)], blood and extracellular fluid base excess [Base (B) and Base (ECF)], and anion gap (AG) were calculated by the machine from pre-programmed algorithms. Methods used for determination of for PaCO2, PaO2, HCO3- (P), HCO3- (P, st), Base (B) and Base (ECF) met the guidelines of the Clinical and Laboratory Standards Institute. Reference intervals were determined with the non-parametric or the standard parametric method dependent upon data distribution.

## Results Reference intervals were determined for pH: 7.37 - 7.49, PaCO2: 4.84 - 7.20 kPa (36.3 – 54.0 mmHg), PaO2: 11.01 - 14.97 kPa (82.6 - 112.3) mmHg, Na+: 133 - 141 mmol L-1, K+: 3.05 - 4.65 mmol L-1, Ca2+: 1.34 - 1.72 mmol L-1, Cl-: 100 - 110 mmol L-1, HCO3- (P): 23.55 - 33.90 mmol L-1, HCO­3- (P, st): 23.87 - 32.45 mmol L-1, Base (B): 0.51 - 8.80 mmol L-1, Base (ECF): -0.53 - 9.39 mmol L-1 and AG: 1.5 - 11.5 mEq L-1.

## Conclusions and clinical relevance These data were derived from the largest group of horses reported in a single study and may aid in interpretation of arterial blood gas, acid-base and electrolyte measurements in clinical practice.

## *Keywords* blood gas analysis, clinical pathology, horse, reference values

## Introduction

Arterial blood gas (ABG) analysis is established in many equine hospitals as an essential tool in managing anaesthetized and critically ill horses (Magdesian 2004; McKenzie 2008). Reference ranges for blood gas and acid-base values for adult horses can be found in anaesthesia and medicine text books, however several resources quote the same data source derived from a small number of experimental animals (McKenzie 2008; Parry 2009),[3] or the source of the values quoted is not given (Mair 2010). Studies investigating the effect of exercise or anaesthesia frequently utilise venous blood samples (Aguilera-Tejero et al. 2000; Viu et al. 2010), or, where arterial blood analysis is performed, conscious baseline values may not be reported (Seaman et al. 1995; Wettstein et al. 2006). Robust justification for accepting currently published values as applicable to a wider, clinically relevant population is therefore lacking.

A reference interval is established to represent a proportion of 95% of the population with a defined confidence level. The Clinical and Laboratory Standards Institute recommend that reference intervals for nonparametric data sets are established using a non-parametric ranking method requiring a minimum of 120 individuals (Horowitz 2008); the parametric method is acceptable for parametric data sets with no minimum required number. With both methods, 90% confidence intervals (CI) for reference interval limits must be established (Horowitz 2008). Commercial diagnostic laboratories are well placed to derive reference intervals for many clinically valuable analytes, however the acute care nature of ABG analysis and labile nature of some of the analytes of interest prevents the analysis being conducted in large laboratories.

This study describes reference intervals for ABG, acid-base and electrolyte values derived from a large population of systemically healthy horses 1 year of age or older presenting for elective surgical procedures requiring general anaesthesia. As secondary outcomes, correlation between barometric pressure and ABG values, and the effect of pre-operative fasting on electrolytes and base excess were investigated.

# Materials and Methods

Following institutional ethical committee approval (VREC154) details of ABG analyses collected as part of a separate study (RETH000379) or for clinical reasons, with owner consent, were collated. Analyses were included if derived from systemically healthy horses, greater than 1 year of age, presented for elective surgical procedures requiring general anaesthesia at The Philip Leverhulme Equine Hospital, University of Liverpool, UK. Horses were considered systemically healthy if graded ASA (American Society of Anesthesiologists Physical Status Classification System) I or II, based on clinical examination and history at presentation. Additionally, samples had to have been acquired prior to administration of any preanaesthetic medication, using minimal restraint (head collar) whilst the horse was in its stable and breathing ambient air. All samples were collected anaerobically into pre-heparinised syringes (Pico50 Arterial Blood Sampler Syringe. Radiometer Medical, Denmark) by direct needle puncture of the transverse facial or common carotid artery by the same investigator. A volume of 2 mL blood was collected over 2-3 breath cycles and samples were analysed immediately using an automated bench top blood gas analyser (ABL 77 Series Blood Gas analyser. Radiometer Medical, Denmark). This analyser utilises multi-use disposable cassettes housing microelectrodes which perform potentiometric measurement of pH, partial pressure of carbon dioxide (PCO2) and concentrations of the electrolytes: sodium (Na+), potassium (K+), calcium (Ca2+) and chloride (Cl-) and amperometric measurement of partial pressure of oxygen (PO2). Actual and standardised plasma bicarbonate concentration [HCO3-(P) and HCO3-(P, st)], blood and extracellular fluid base excess [Base (B) and Base (ECF)] and anion gap (AG) are calculated by the machine from pre-programmed algorithms. Correction for patient body temperature was not performed. Automatic two-point calibration of all sensors was performed every 4 hours and following installation of a new calibration solution pack, allowing sensitivity, electrical and thermal stability and electrical range to be verified. In addition, a daily external quality control was performed using commercially available tonometered reference solutions (Radiometer QUALICHECK+ quality control ampoules. Radiometer Medical, Copenhagen, Denmark). Barometric pressure was recorded at the time of analysis. Printed analyser outputs were collected and values for the above variables transferred to an Excel spreadsheet.

## Statistics

Distribution of data was assessed using visual inspection of histograms, Q-Q plots and the Kolmogorov-Smirnov test. Reference intervals were determined with the non-parametric or the standard parametric method depending on distribution, with 90% CI (Reference Value Advisor V2.1 add-in for Microsoft Excel, France; Geffre et al. 2011). Dixon-Reed’s and Tukey’s tests were used to identify outliers. All other statistics were calculated using the commercial statistics programme IBM SPSS (version 24; IBM Corp, Armonk, NY, USA). The paired T-test and the Mann-Whitney U test were used to evaluate differences in Base (B), Base (ECF) and electrolytes between horses withheld food and horses fed prior to sample collection. Statistical significance was assumed at *p*< 0.05. Results are presented as mean ± standard deviation (SD) or median (range), dependent upon data distribution.

# Results

Results of ABG analysis from 139 horses (87 geldings, 46 mares and 6 entire males), acquired over a 36-month period were included. Main represented breeds were Thoroughbred and Thoroughbred crosses (19.5%), warmblood and warmblood crosses (17.2%), cobs (11.5%) and Irish Sports Horses (10.8%). Age and bodyweight distributions were 9 (1-22) years and 554 ± 91.2 kg respectively. Most surgeries (*n* = 98) were orthopaedic procedures; 39 arthroscopies, 22 tenoscopies, 21 neurectomy-fasciotomies or desmotomies, 7 podiatry procedures and 9 miscellaneous. Soft tissue surgeries (*n* = 41) consisted of 21 cutaneous mass removals, 7 upper airway surgeries, 5 ocular procedures, 4 dental extractions and 4 castrations.

A total of 31 ABG samples were obtained by carotid artery puncture, 108 from the transverse facial artery. Comparison of results from both sampling sites identified no statistically, or clinically, significant differences, therefore results were combined for analysis.

Distribution, reference intervals and 90% CI for reference interval limits for pH, PaCO2 (arterial partial pressure of carbon dioxide), PaO2 (arterial partial pressure of oxygen), Na+, K+, Ca2+, Cl-, HCO3-(P), HCO3-(P, st), Base (B), Base (ECF) and AG are given in Table 1. No outliers were identified with Dixon-Reed’s test. Tukey’s test identified no outliers for PaCO2 and PaO2, seven outliers for AG and between one and four outliers for the remaining analytes. The outliers were retained for the estimation of the reference intervals as no reasons for aberrant observations were found when the individual records were checked (Horowitz 2008). The methods by which the reference intervals were determined for PaCO2, PaO2, HCO3- (P), HCO3- (P, st), Base (B) and Base (ECF) met the guidelines of the Clinical and Laboratory Standards Institute (Horowitz 2008). The remaining variables did not meet the guidelines due to the 90% CI exceeding the recommended width, of no more than 20% of the reference interval (Harris & Boyd 1995). For Na+, Cl-, K+, Ca2+ and AG either the upper or lower limit exceeded 20% of the reference interval and for pH both upper and lower limits were wider than recommended (Table 1).

Barometric pressure (mean ± SD) was recorded as 100.77 ± 1.28 kPa (755.8 ± 9.6 mmHg), with a range of 97.41 - 103.61 kPa (730.6 - 777.1 mmHg). No correlation was found between barometric pressure and PaO2 (Pearson correlation coefficient 0.09, *p* = 0.3), and a weak correlation was found between barometric pressure and PaCO2 (Pearson correlation coefficient -0.249, *p* = 0.003). Barometric pressure recordings were unavailable for two cases. A weak correlation was found between age and PaO2 (Pearson’s correlation coefficient 0.232, *p* = 0.011).

At the time of sample collection, 100 horses had undergone a period of food deprivation, 20 had access to food and for 19 horses this could not be determined. Horses which had food withheld had higher Base (B) (*p* < 0.01), Base (ECF) *(p* < 0.01) and lower K+ (*p* < 0.01), Ca2+ (*p* < 0.01) and Cl- (*p* = 0.010) values (Table 2). A weak correlation was found between K+ and Base (B) and Base (ECF) (Spearman correlation -0.199, *p* = 0.019*;* -0.198, *p* = 0.020 respectively).

# Discussion

This study reports ABG, electrolyte and acid-base variables derived from a large group of systemically healthy horses obtained under clinical conditions. The largest group of ABG samples from horses evaluated previously consisted of 43 samples and only reported PaCO2, PaO2, pH, HCO3- (P) and Base (B) (Nolte et al. 1982). Blood-gas and electrolyte measurements are taken frequently in clinical practice to gauge disease severity, guide fluid therapy and direct ventilation strategies under anaesthesia (Hubbell & Muir 2015). The reference intervals developed from this population will facilitate interpretation of blood-gas and electrolyte results in clinical practice and in future research. It is also the only study meeting the guidelines of the Clinical and Laboratory Standards Institute for establishing reference intervals for equine arterial PCO2, PO2, HCO3- (P), HCO3- (P, st), Base (B) and Base (ECF).

The reference intervals derived in this study vary from those previously reported for the variables PaCO2, PaO2, K+ and Base (B and ECF) (Rose et al. 1979; Aguilera-Tejero et al. 1998; Meyer et al. 2010). The upper end of the reference interval for PaCO2 is 7.20 kPa (54.0 mmHg) which is higher than anticipated given commonly utilised text book reference ranges of 35-45 mmHg (Mair 2010) and 34-50mmHg (Parry 2009). Most previously reported values are from Thoroughbred and Standardbred horses, whilst most of this hospital caseload consists of pleasure horses used for low intensity exercise. Studies in human athletes suggest that athletic training leads to higher resting PaCO2 (Thomas et al. 2013), whilst in horses the level of physical fitness does not appear to impact on resting PaCO2 values (Roberts 1999). A delay in sample analysis can result in an increase or decrease in measured PaCO2dependent upon cell metabolism and diffusion of gases through the syringe material (Knowles et al. 2006). However, all samples were analysed within 5 minutes of acquisition, a duration shown not to significantly alter measured PaCO2 in plastic syringes (Picadent et al. 2007).

Stopyra et al. (2012) found PaCO2 in 18 horses with equine asthma to be 53.5 ± 5.7 mmHg, a mean value similar to the upper end of this range. There were no clinical signs of respiratory disease within this study population, but subclinical disease may have been present. Bracher et al. (1991) reported a prevalence of subclinical equine asthma of 54% in a random population of Swiss horses. PaCO2 data are not reported, but the authors state there were no significant differences in arterial blood gas analysis between healthy horses and those with subclinical or mild respiratory disease.

The reference interval defined for PaO2 is wider than previously reported ranges (Rose et al. 1979; Aguilera-Tejero et al. 1998). PaO2 can be influenced by many factors including barometric pressure, inspired oxygen fraction, ventilation-perfusion mismatch, hypo- or hyperventilation and alveolar diffusion barrier impairment (Hubbell & Muir 2015). The lower values in the range are consistent with those previously reported in ponies (Mauderley 1974), but lower than most other reported values in adult horses. The upper end of the range is similar to previously published work (Milne et al. 1975; McMurphy & Cribb 1989). Equine haemoglobin has a higher affinity for oxygen than human haemoglobin, as indicated by their relative P50 values (23.8 and 26.6 mmHg respectively) with PaO2 of 73.7 mmHg corresponding to an SpO2 of 95% (Clerbaux et al. 1986). Consequently, equine haemoglobin is almost completely saturated with oxygen above a PaO2 of 70-80 mmHg (Hubbell & Muir 2015).

An age effect on PaO2 has previously been reported, producing a variation between old and young horses of approximately 10mmHg (Aguilera-Tejero et al 1998). Only a weak correlation was found between age and PaO2 in this study population, which is unlikely to be of clinical significance.

Most studies do not report barometric pressure in conjunction with PaO2 values, unless the effect of changing altitude is being investigated and we were interested in how much barometric pressure variation affected measured PaO2. The study was conducted 63 meters (206 feet) above sea level and barometric pressure varied over the study period (97.41 - 103.61 kPa (730.6 - 777.1 mmHg)). Using the alveolar gas equation, the difference in ideal alveolar gas oxygen tension, and hence potentially in PaO2 values, between the highest and lowest barometric pressures recorded in this study would be 1.29 kPa (9.7 mmHg). No correlation was found between barometric pressure and PaO2 suggesting that normal variations in barometric pressure at fixed altitude have little influence on PaO2 and its effect can be discounted. A weak correlation was found between barometric pressure and PaCO2, this is not considered clinically significant.

Whilst all PaO2 values obtained were confirmed to be physiologically plausible given the sampling circumstances, the possibility that a small volume of air could have been entrained during sample acquisition cannot be excluded, falsely elevating measured PaO2 (and decreasing PaCO2). The derived reference interval for PaO2, although wider than expected, likely reflects normal physiologically appropriate variation consistent with the altitude and barometric pressures at which samples were obtained.

The reference interval for K+ is lower than expected, based upon previously reported ranges (Aguilera-Tejero et al. 1998; Meyer et al. 2010). The arterial samples in this study were collected either the day before, or on the day of surgery prior to any drug administration. Horses which had food withheld before sampling had a significantly lower potassium concentration than those fed up to the time of sample collection. Reduced dietary intake has not been commonly described to cause a reduction of plasma potassium concentration in horses (Fielding 2015), and Freestone et al. (1991) found a 72-hour period of food withhold did not significantly alter plasma potassium concentration. It is unlikely therefore that the short period of fasting (< 12 hours) in this population would directly reduce plasma potassium concentration. The stress induced by withholding food from a stabled horse may lead to increased release of catecholamines such as epinephrine, promoting the translocation of potassium into cells, therefore reducing the measured plasma concentration (Medica et al. 2017). B-adrenergic receptor stimulation has been demonstrated to reduce plasma potassium concentration via increased cellular uptake in the dog, cat and rat (Struther & Reid 1984). This mechanism may account for the lower than expected potassium reference range. Previously reported mean arterial K+ concentration in horses range from 3.8 to 4. 5 mmol L-1 (Aguilera-Tejero et al. 1998; Meyer et al. 2010), values which lie within the reference interval derived from this study population; however, it is likely that the reference interval is lower than if it had been derived entirely from horses with unrestricted access to food. Calcium and chloride values in the study population were marginally lower in the group from which food had been withheld, which may also have had an impact on these reference intervals. Electrolyte concentrations for venous blood are widely available, and the arterial concentrations derived in this study are in broad agreement with these, except for potassium (Meyer et al. 2010; Peiro et al. 2010).

Base excess indicates the metabolic component of acid-base balance. Two forms are commonly reported by blood gas analysers, Base (B) and Base (ECF), reflecting the different buffering capabilities of blood alone, or the whole ECF. Rose et al. (1979) found Base (B) in healthy endurance horses to be 1.1 ± 1.4 mmol L-1 and Art & Lekeux (1995) found it to be 1.2 ± 0.7 mmol L-1 in healthy Standardbreds. By comparison, Base (B) in this population was 4.14 ± 2.35 mmol L-1 with a reference interval of -0.51 - 8.80 mmol L-1. This is broader and more alkalotic than expected based on previous reports. Base excess is influenced by alterations in bicarbonate concentration due to renal compensation for chronic respiratory acid-base disturbances. Therefore, the higher than expected range of PaCO2 may have influenced the base excess findings. However, as HCO3- values were not high this is unlikely. It is known that diet influences metabolic acid-base status (Nagy et al. 2003). The values reported in this equine population are broadly comparable to those reported in other herbivores which utilise a fermentative digestive system (Nagy et al. 2003; Eatwell et al. 2013). Base (B and ECF) were higher in horses which had food withheld prior to sample collection. Given this difference it is important to consider feeding status when interpreting acid-base balance and electrolytes. In the non-fasted group, the Base (B) and Base (ECF) distributions were still wider and more alkalotic than previously reported values (Rose 1979; Art & Lekeux 1995), but care must be taken in interpretation of this data due to the small group size. Metabolic alkalosis can reduce the plasma potassium concentration by translocation of plasma potassium into cells in exchange for hydrogen ions (Adrogue & Madias 1981). A statistically significant, but very weak negative correlation between arterial K+ concentration and Base (B) and Base (ECF) was detected, therefore the higher base excess values in the fasted group likely had little influence on the plasma potassium concentration in this group.

The reference intervals were established with the parametric method and the non-parametric ranking method based upon data distribution. Other methods of establishing reference intervals include the robust method which is recommended if the sample size is insufficient to use the aforementioned methods; but is more prone to error if the data are not symmetrically distributed (Friedrichs et al. 2012). It is recommended that the width of the 90% CI for a reference limit should be less than 0.2 times the width of the reference interval (Harris & Boyd 1995). This is a text book recommendation with no described justification. The CI for the non-parametric variables (pH, Na+, Cl-, K+, Ca2+, AG) studied are wider than recommended indicating that a larger study population is required. The width of both CI for pH, and the lower limit CI for Na+ and Ca2+, are only marginally wider than the recommendation at 25-31% of their reference intervals. The remaining wide CI vary between 50 and 75% of their reference interval widths. The retention of outlying values for determination of the reference intervals may have led to this finding. The guidelines provided by the Clinical and Laboratory Standards Institute state that unless outliers are known to be aberrant observations the values should be retained (Horowitz 2008). On re-examining the case details of horses found to be outliers, no reason for exclusion could be found and they were therefore retained within the dataset. Re-analysis of the data without the outliers did not substantially reduce the width of the confidence intervals, therefore the increased width is likely due to data distribution rather than outlying data points.

Several limitations of this study must be recognised. Hospital protocol was to withhold food for 8 - 12 hours prior to induction of general anaesthesia and timing of sample acquisition in relation to general anaesthesia was not standardized. Consequently, there is a variation in the length of time food was withheld at time of sampling from 0 - 12 hours and this seems to have been significant in influencing base excess, K+, Ca2+ and Cl- and further research investigating this is warranted. Although the study population was deemed healthy based upon clinical examination it is possible that mild or subclinical respiratory disease was present in some cases affecting PaCO2 and PaO2 via ventilation and perfusion matching and alveolar diffusion barrier mechanisms (Littlejohn & Bowles 1992). Obtaining the arterial sample may also have caused stress in some individuals leading to changes in respiratory pattern and arterial blood pressure, both of which could impact on blood gas partial pressures. This may explain the lack of correlation detected between PaO2 and barometric pressure or age in this study.

Analyses were not corrected for body temperature. There is no consensus on the necessity for temperature correction of blood gas analysis, however there is increasing support for the view that it is unnecessary in many clinical situations (Ashwood et al 1983). Previous studies report correction to pulmonary artery temperature (Art & Lekeux 1995); rectal temperature (Aguilera-Tejero et al. 1998; Meyer et al. 2010); do not stipulate site of temperature determination (McMurphy & Cribb 1989); or do not specify whether temperature correction was performed (Mauderley 1974; Milne et al 1975). The analyser in this study measures samples at a temperature of 37 °C. Normal equine rectal temperature is 36.5 - 38.5 °C (Rose & Hodgson 1993). In this healthy population, any deviations from 37 °C should have been minimal (± ~ 1 oC). Ashwood et al. (1983) recommend temperature correction of pH and PCO2 is only advisable when patient temperature deviates from 37 oC by > 2 oC and for PO2 only when by >1 oC. Commercial blood gas analysers utilise temperature correction algorithms derived in human studies and these have not been validated in equine blood. Fedde (1991) calculated correction factors for pH, PO2 and PCO2 for equine blood and stated they were similar to human values, but only considered a temperature range of 37 – 41 oC. Using Fedde’s correction factors, this would give variation in PaO2 and PaCO2 of approximately ± 2 mmHg and ± 0.014 pH from measured values, this is of little clinical significance.

The Clinical and Laboratory Standards Institute guidelines for establishment of reference intervals are intended for use by laboratories handling human samples and may not be directly translatable to veterinary species. There is, however, no veterinary equivalent of these guidelines.

## Conclusions

The above study provides clinically relevant reference ranges (not corrected for body temperature) for equine ABG, acid-base and electrolyte variables and represents the largest cohort of clinically healthy adult horses in a single study. It is also the only study meeting the guidelines of the Clinical and Laboratory Standards Institute for establishing reference intervals for equine arterial PaCO2, PaO2, HCO3- (P), HCO3- (P, st), Base (B) and Base (ECF). The data are intended to contribute to the ever-growing resource of evidence based veterinary medicine for both direct clinical application and further research.

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# Tables

**Table 1** Reference intervals for arterial blood gas, acid-base and electrolyte values derived from 139 healthy adult horses. Samples were obtained under conditions of minimal restraint, from conscious, standing animals breathing room air and prior to administration of any pre-anaesthetic agents. Distributions presented as median (range) or mean ± standard deviation. CI = Confidence Interval.

\* CI wider than 20% of the reference interval.

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| --- | --- | --- | --- | --- |
| **Variable** | **Distribution** | **Reference Interval** | **90% CI lower limit** | **90% CI upper limit** |
| **pH** | 7.42 (7.35 - 7.49) | 7.37 - 7.49 | 7.35 - 7.38\* | 7.46 - 7.49\* |
| **PaCO2 (kPa) [mmHg]** | 6.02 ± 0.59 (4.40 - 7.33) [45.2 ± 4.4 (33.0 - 55.0)] | 4.84 - 7.20 [36.3 – 54.0] | 4.71-4.98 [35.4 - 37.3] | 7.06-7.33 [52.9 - 55.0] |
| **PaO2 (kPa) [mmHg]** | 13.00 ± 1.00 (10.40 - 15.86) [97.5 ± 7.5 (78.0 - 119.0)] | 11.01 - 14.97 [82.6 - 112.3] | 10.80 - 11.24 [81.0 - 84.3] | 14.74 - 15.20 [110.5 - 114.0] |
| **Na+ (mmol L-1)** | 137 (132 - 144) | 133 - 141 | 132 - 134\* | 139 - 144\* |
| **K+ (mmol L-1)** | 3.60 (2.80 - 5.40) | 3.05 - 4.65 | 2.80 - 3.10 | 4.20 - 5.40\* |
| **Ca2+ (mmol L-1)** | 1.54 (1.30 - 1.73) | 1.34 - 1.72 | 1.30 - 1.42\* | 1.69 - 1.73 |
| **Cl-  (mmol L-1)** | 104 (100 - 113) | 100 - 110 | 100 - 101 | 108 - 113\* |
| **HCO3-(P) (mmol L-1)** | 28.73 ± 2.61 (21.40 - 37.70) | 23.55 - 33.90 | 22.99 - 24.14 | 33.28 - 34.49 |
| **HCO3-(P,st) (mmol L-1)** | 28.16 ± 2.16 (22.00 - 36.30) | 23.87 - 32.45 | 23.40 - 24.35 | 31.94 - 32.95 |
| **Base(B) (mmol L-1)** | 4.14 ± 2.35 (-2.90 - 12.50) | -0.51 - 8.80 | -1.02 - 0.02 | 8.24 - 9.34 |
| **Base(ECF) (mmol L-1)** | 4.43 ± 2.50 (-3.00 - 13.40) | -0.53 - 9.39 | -1.08 - 0.03 | 8.80 - 9.96 |
| **Anion Gap (mEq L-1)** | 7.2 (-1.7 - 12.1) | 1.5 - 11.5 | -1.7 - 3.5\* | 10.4 - 12.1 |

**Table 2** Comparison of blood and extracellular fluid base excess [Base (B) and Base (ECF)] and electrolyte values between horses which had undergone a period of food deprivation and those which had not, prior to arterial blood sampling. Data presented as median (range) or mean ± standard deviation. Statistical significance assumed at *p* < 0.05.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Food withheld** | **Food not withheld** |  |
|  | ***n*= 100** | ***n*= 20** |  |
| **Base(B) (mmol L-1)** | 4.41 ± 1.99 | 2.49 ± 2.44 | *p* = 0.0002 |
| **Base(ECF) (mmol L-1)** | 4.71 ± 2.14 | 2.72 ± 2.55 | *p* = 0.0004 |
| **Na+ (mmol L-1)** | 137 (132 - 144) | 137 (134 - 141) | *p* = 0.84 |
| **K+ (mmol L-1)** | 3.55 (2.80 - 5.50) | 3.90 (3.10 - 5.40) | *p* < 0.0001 |
| **Ca2+ (mmol L-1)** | 1.53 (1.30 - 1.71) | 1.63 (1.46 - 1.73) | *p* < 0.0001 |
| **Cl- (mmol L-1)** | 104 (100 - 110) | 107 (101 - 113) | *p* = 0.01 |