BRIEF COMMUNICATION

Blood gas and serum biochemical RIs for healthy newborn Murrah buffaloes (*Bubalus bubalis*)

Running Header: Blood biochemical profile in buffalo calves

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Background: There is a lack of published work on RIs for newborn buffaloes. Establishing blood gas and serum biochemical RIs for newborn buffaloes is important for monitoring health.

Objectives: This study establishes blood gas and serum biochemical RIs of newborn buffaloes.

Methods: Twenty-eight newborn buffaloes, 10-30 days old, were selected. Thirty blood biochemical variables were analyzed. The Anderson–Darling test was used to assess the normality of the distribution. The Dixon test and the Tukey test were used to identify outliers. The RI and 90% CI were determined using standard and robust methods and the Box–Cox transformation.

Results: A total of 30 RIs for healthy buffalo calves have been reported in this study. RIs for blood gas variables were reported for pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), saturation of O_2 (SO_2), bicarbonate ($cHCO_3^-$), base excess (BE), total carbon dioxide ($ctCO_2$) and anion gap (AG). RIs for serum biochemical variables were reported for glucose (GLU), direct bilirubin (DB), total bilirubin (TB), AST, ALP, GGT, CK, LDH, creatinine (CREA), urea, cholesterol (CHOL), triglycerides (TG), Ca, P, Mg, Na, K, iCa, Cl, iron, total protein (TP) and albumin (ALB).

Conclusions: This is the first reported work covering complete serum chemistry and blood gas RIs for healthy one-month-old Murrah buffaloes.

Keywords: bicarbonate, clinical biochemistry, diagnosis, neonatal, prognosis, water buffalo

Water buffalo (*Bubalus bubalis* of the subfamily Bovinae) are distributed throughout Brazil, with 1.5 million animals estimated in 2014. While the North and Northeast regions contain the largest number of animals (79.2%) specialized for meat production, the Southeast region (10.2% of all animals) has the highest production of buffalo milk, with the Murrah and Jafarabadi buffalo being the most important breeds.[1]

In healthy one-month-old animals, serum biochemical profile studies[2,3] have been conducted in bovines, but very rarely in buffaloes.[4] Studies of blood gas variables have also been performed with healthy one-month-old bovine calves[5,6] but not with buffalo calves, for which only pH has been reported.[4] Additionally, studies clearly show that the use of adult serum biochemical RIs for assessing calves is not appropriate, since many alterations occur with advancing age.[4,7] Therefore, the establishment of RIs for blood gas and serum biochemical variables from healthy one-month-old buffalo calves is of great importance.

This study aimed at establishing baseline RIs for blood gas and blood serum biochemical variables in healthy neonatal buffalo calves to have a reference when evaluating pathologic conditions.

This research was approved by the Ethics Committee on Animal Use at the School of Agricultural and Veterinary Sciences (FCAV), São Paulo State University, Jaboticabal-SP, Brazil (Protocol number: 010885-08). The calves used in the study were from commercial herds located in São Paulo state from adult female Murrah buffaloes used in milk production. Calves were kept together with their lactating mothers, which were housed in a semi-intensive system on a roughage diet. During the rainy season (summer), the lactating buffaloes were kept on pasture and supplemented with a protein concentrate. During the dry season (winter), the lactating buffaloes were fed chopped sugar cane and a supplemented protein concentrate. Calves were fed with fresh buffalo milk and had access

74 to commercial feed (Fri-Vitelina 18/70 with 18% crude protein, Fri-Ribe, Nutreco Brasil
75 Nutrição Animal LTDA., Mirassol, SP, Brazil), hay and water ad libitum.

76 The health status of 35 Murrah buffalo calves, 10 to 30 days old, was verified by a
77 physical examination, and confirmation of appropriate passive immunity transfer. Their
78 feces were analyzed for signs of diarrhea, blood, and mucus. Fecal consistency scores
79 were determined as 0, normal (firm); 1, mild diarrhea (soft); 2, and moderate to severe
80 diarrhea (liquid). [8] The degree of dehydration was estimated as 0, absent (normal skin
81 turgor and bright eyes); 1, mild (skin turgor slightly decreased and eyes not retracted); 2,
82 moderate to severe (skin turgor decreased and eyes retracted). [8] Rectal body temperature
83 was also measured.

84 Inclusion criteria for study calves were firm feces (score 0), rectal temperature <
85 40.0°C, normal skin turgor, and bright eyes (dehydration score of 0). In addition,
86 appropriate passive immunity transfer defined as an IgG > 10.0 g/L as measured by sodium
87 dodecyl sulfate-polyacrylamide gel electrophoresis[9] (Densitometer CS-9301PC, Shimadzu
88 Corporation, Tokyo, Japan) and a total protein (TP) > 5.5 g/dL as measured by the Biuret
89 method (Labquest semi-automatic analyzer, Labtest Diagnostic, Lagoa Santa, MG, Brazil)
90 were also required.

91 All blood samples were collected during the dry season of the year (winter, between
92 June and August) when the lactating buffaloes were fed chopped sugar cane and a
93 supplemental protein concentrate. Only one blood sample was collected from each calf by
94 venipuncture using a vacuum collection system after application of a local antiseptic
95 (iodized alcohol).

96 The blood samples were collected into siliconized plastic tubes containing fluoride
97 oxalate as an additive (4.0 mL, 13 x 75 mm vacutainer tube, Becton Dickinson Vacutainer,
98 Franklin Lakes, NJ, USA) and were centrifuged immediately, on-site, at 1,000*g* for 5 min

to obtain the plasma necessary to analyze glucose (GLU) concentrations. The plasma was stored on ice and processed within 1 to 2 h after collection. Serum was also collected into siliconized plastic tubes without anticoagulant (10.0 mL, 16x100 mm vacutainer tube, Becton Dickinson Vacutainer, Franklin Lakes, NJ, USA) for biochemical profile analysis using a Labquest semi-automatic analyzer (Labtest Diagnostic, Lagoa Santa, MG, Brazil) and an electrolyte analyzer (Roche Diagnostics GmbH, Mannheim, Germany). These samples were also centrifuged immediately, on-site, at 1,000*g* for 10 min, and 1.5 mL aliquots of serum were taken and stored in Eppendorf tubes, previously identified, and frozen (-20°C) until analysis was performed, which was not more than one month after collection.

For analysis of blood gas variables, a Roche OMNI C (Roche Diagnostics GmbH, Mannheim, Germany) was used. Blood samples were collected from the jugular vein using 1.0 mL plastic syringes containing sodium heparin as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were stored on ice and processed within 1 to 2 hours after collection. At the time of analysis, blood gas variables were corrected by entering the rectal temperature in the electronic apparatus panel of the Roche OMNI C.

For serum biochemical analysis, the following variables were measured: serum GLU concentrations, direct bilirubin (DB), total bilirubin (TB), creatinine (CREA), urea, cholesterol (CHOL), triglycerides (TG), Ca, P, Mg, Cl, iron, TP, and albumin (ALB) and serum activity of AST, ALP, GGT, CK, LDH. All reagents were obtained from the analyzer manufacturer (Labtest Diagnostic, Lagoa Santa, MG, Brazil). The readings were performed using a semi-automatic spectrophotometer with specific wavelengths for each constituent. The wavelengths and test principles are listed in Table 1. A 9180 electrolyte analyzer (Roche Diagnostics GmbH, Mannheim, Germany) was used to measure the serum concentrations of Na, K and ionized Ca (Table 1).

For blood gas variables, the following were measured: pH, partial pressure of oxygen (pO_2); partial pressure of carbon dioxide (pCO_2); total carbon dioxide ($ctCO_2$); bicarbonate $(cHCO_3)^-$; AG, saturation of oxygen (SO_2); and base excess (BE). The test principles are listed in Table 1.

Multi-analyte controls Combitrol TS+ Level 1, 2, and 3 (Roche Diagnostics GmbH, Mannheim, Germany), and Qualitrol 1H and 2H (Labtest Diagnostic, Lagoa Santa, MG, Brazil) were used to ensure internal quality control for analyzing biochemical and blood gas variables. Quality control assessment was always performed before analyzing each analyte, which only occurred when quality control procedures were acceptable (standard sample concentrations within the expected range and with coefficients of variation less than 5%). After ensuring internal quality control, each buffalo serum sample was measured in duplicate for each analyte. Duplicate results were only used when intraassay coefficients of variation were less than 10%.

The RIs were determined according to the guidelines of the American Society for Veterinary Clinical Pathology (ASVCP) (sample size: $20 \leq x < 40$).[10] The Anderson–Darling test was used to assess the normality of the distribution with $P<.05$ (Reference Value Advisor). [11] The Dixon test and the Tukey test (3xIQR, 1.5xIQR) were used to identify outliers and suspect outliers (Reference Value Advisor).[11] The RI and 90% CI for the lower and upper limits were determined using untransformed data when the data distribution was Gaussian (Standard) or symmetric but not Gaussian (Robust). When the distribution was not symmetric, the Box–Cox transformation was performed to normalize the data (Reference Value Advisor).

From the 35 animals that were examined, 7 were excluded due to IgG concentrations <10.0 g/L. Therefore, 28 healthy Murrah buffalo calves, 10 to 30 days old,

148 weighing an average of 52.1 kg (Table 2) and meeting the inclusion criteria, constituted the
149 experimental group.

150 Since the study included 28 newborn buffaloes, RIs were determined using the
151 Reference Value Advisor[11] according to the ASVCP guidelines.[10] Suspect outliers were
152 identified for pH (2 suspect Tukey for minimum value), total carbon dioxide ($ctCO_2$) (2
153 suspect Tukey for minimum value) and CK (2 suspect Tukey for minimum value). The
154 calves were determined to be healthy by moderately stringent criteria, and no sample
155 quality abnormalities such as hemolysis or other alterations that could justify exclusion
156 from were observed. Additionally, all preanalytic procedures were performed correctly,
157 and sample processing was carried out in a standardized manner using methodologies that
158 are already well documented in the literature, with reagents stored appropriately and with
159 devices in an adequate operational state. In light of these considerations, the suspect
160 outliers were retained in the data set for analysis.

161 All variables were analyzed in 28 samples except Na, K and iCa (only 27 samples
162 due to insufficient sample volumes for one animal) and saturation of O_2 (SO_2) (only 23
163 samples due to a device error in 5 samples).

164 The results of the statistical analysis, including RIs for serum biochemical variables
165 and blood gas variables, are presented in Tables 3 and 4, respectively.

166

167 **Discussion**

168

169 This study represents the first published report of complete biochemical and blood gas RIs
170 in neonatal buffalo calves. To our knowledge, no RIs have been reported for buffaloes
171 during the first month of life for 9 of the serum biochemical variables reported in our
172 study, namely, concentrations of DB, CHOL, TG, Na, K, iCa, and Cl and activities of CK

and LDH. Among the 8 blood gas variables measured, only mean and standard deviation of pH had previously been reported in neonatal buffaloes, but not the RI[4].

Data on serum CREA concentrations were previously reported in 0-, 15-, 30-, and 45-day-old buffalo calves raised in Brazil[4] and the results were higher than those reported here (Table 3), which was probably due to the different methodologies used, such as the Lustosa-Basques method[4] while we used the alkaline picrate-Jaffé method. Serum CREA concentrations decreased when comparing 0-day-old buffalo calves with 15-, 30-, and 45-day-old buffalo calves, but were similar among the 15-, 30-, and 45-day-old buffalo calves[4]. Therefore, according to the literature[12], CREA concentrations for calves older than one week are likely similar to those determined for adult cattle.

Serum GGT activity can be markedly increased in newborns due to colostrum ingestion, as reported in bovines[2] and buffaloes[4], while with advancing age, this activity decreases due to degradation of the GGT in colostrum.[4,7] Serum GGT activity was previously reported in 0-, 15-, 30-, and 45-day-old buffalo calves, with respective average serum activities of 729.52 IU/L, 158.82 IU/L, 37.54 IU/L, and 25.69 IU/L.[4] In our study, in which the mean age of the animals was 18.8 days, the serum GGT activity (64.9 IU/L) was between the values for 15- and 30-day-old buffalo calves in the study above.[4]

Serum ALP activity can be increased in one-day-old newborns, and is probably due to tissue release and not colostral ingestion and absorption of this enzyme.[2] Serum ALP activity decreases with age, which was previously reported in 0-, 15-, 30-, and 45-day-old buffalo calves, with respective average serum activity of 388.26 IU/L, 288.63 IU/L, 209.32 IU/L and 169.02 IU/L[4]. In our study, the mean ALP activity was 188 IU/L in 18.8 day-old buffalo calves, which fell between the ALP activities for 30- and 45-day-old buffalo calves in the previous report.[4] As the methodology can have an impact on enzymatic test results this fact should be considered when comparing data from different studies.

198 Serum TB and P concentrations and serum AST activities were also previously
199 reported in 30-day-old buffalo calves[4], and the results were again lower than those reported
200 in our work. Studies performed in Brazil on buffaloes[4] showed that serum TB
201 concentrations are high after birth and decrease with time. In a previous study[4], TB
202 decreased significantly in buffalo calves, from 9.6 μmol/L (day of birth) to 5.6 μmol/L (30
203 days after birth). The higher TB concentrations at birth can be caused by decreased
204 efficiency in the bilirubin excretion mechanisms by the placenta, low UDP-
205 glucuronyltransferase activity in the liver, and high β-glucuronidase concentrations in the
206 intestine, which have all been cited as causing high TB concentrations in human
207 neonates.[13]

208 Plasma GLU concentrations have been previously reported in 30-day-old buffalo
209 calves[4], and the results were similar to those reported in our work. According to the
210 literature[14], GLU levels are higher in young animals because plasma growth hormone (GH)
211 concentrations are higher at this age. As GH is responsible for most hepatic GLU secretion,
212 blood GLU levels will be higher to provide the necessary energy for growth. Therefore, the
213 decrease in GLU levels with advancing age probably reflects a decrease in plasma GH
214 concentrations.

215 Serum urea concentrations were also previously reported in 30-day-old buffalo
216 calves[4], and the results were also similar to our results. In bovines, studies show that serum
217 urea concentrations are affected by age and are higher in adult animals[12]. Lower urea
218 concentrations in younger animals can be caused by an anabolic state, which is typical of
219 rapid growth, and which leads to high fluid consumption and increased urine flow[15].

220 Serum iron concentrations are comparable between the animals in our study and
221 newborn buffaloes (0-45 days old) in another study.[4] It is important to note that iron
222 concentrations are higher in newborn buffaloes than in newborn bovines[4], which may be

223 due to higher iron concentrations in buffaloes colostral secretion compared with bovines.[4]
224 Other variables such as Ca, Mg, TP, and ALB were also previously reported in 30-day-old
225 buffalo calves[4] with similar results to those of our study.

226 Two limitations of our study must be considered when discussing the results
227 presented in our work: 1) the number of samples (*n*=28) and 2) the age range (10-30 days,
228 with a mean age of 18.8 days). The smaller the sample size, the higher the degree of
229 uncertainty in the estimation of RIs. Although nonparametric methods of determining RI
230 are optimal, RIs determined using alternative statistical methods are used when small
231 numbers of reference subjects are available. Therefore, when 20 to 40 reference samples
232 are available, methods that are robust (distribution independent) or parametric (if normality
233 can be established) can be used as an alternative way to calculate RIs and was used in this
234 study.

235 Biochemical profile changes are common in the first month of life when the calf is
236 adapting to extrauterine life.[4] A study that analyzed serum biochemical profiles of buffalo
237 calves from Brazil on the day of birth and at 15, 30, and 45 days after birth verified
238 significant variation of some variables with age, such as TP, TB, GGT, ALP, and Mg.[4]
239 Therefore, during the time we performed this study (10 to 30 days of age), with an age
240 range of 20 days, changes in some variables could occur and must be considered. On the
241 other hand, since very little information has been reported for buffaloes in the first month
242 of life, the data presented in this study may be helpful as a baseline for animals during this
243 period.

244 In conclusion, a total of 22 RIs for serum biochemical variables and 8 RIs for blood
245 gas variables from healthy buffalo calves have been reported and can be used as baseline
246 data to aid in monitoring biochemical alterations during pathologic conditions.
247 Additionally, 9 of the 22 RIs for serum biochemical variables (DB, CHOL, TG, Na, K,

iCa, and Cl, and serum CK and LDH activities), and all 8 blood gas variables (pH, pO_2, pCO_2, $ctCO_2$, $cHCO_3^-$, AG, SO_2, and BE) from these buffalo calves have been reported for the first time in literature.

Acknowledgments

The authors thank the São Paulo Research Foundation (FAPESP) for financial support (process number: #2008/50388-7, #2009/12350-0).

Disclosure: The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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Table 1. Analytic methods for clinical biochemical variables measured in buffalo calves.

Variables	Method	Absorbance required
Glucose	GOD – Trinder [1]	490–520 nm
Direct and total bilirubin	Labtest DCA (Diazotized Dichloroaniline) [1]	530–550 nm
AST	Kinetic UV-IFCC [1]	340 nm
ALP	Bowers and McComb modified [1]	405 nm
GGT	Szasz modified [1]	400–420 nm
CK	Kinetic IFCC [1]	340 nm
LDH	Pyruvate-Lactate [1]	340 nm
Creatinine	Alkaline picrate – Jaffé [1]	500–540 nm
Urea	Enzymatic UV [1]	340 nm
Cholesterol, Triglycerides	Enzymatic Trinder [1]	490–510 nm
Ca	CPC – cresolphthalein [1]	550–590 nm
P	Daly and Ertingshausen modified [1]	340 nm
Mg	Labtest - Magon sulfonated [1]	500–540 nm
Na, K, iCa	Ion-selective electrode (ISE) [2]	-
Cl	Labtest - thiocyanate Mercury [1]	450–510 nm / -
Iron	Goodwin modified [1]	540–580 nm
Total Protein	Biuret [1]	530–550 nm
Albumin	Bromocresol green [1]	600–640 nm
IgG	SDS – PAGE [3]	-
pH	Ion-selective electrode (ISE) [4]	-
pO_2	Clark measuring principle - that is, the measurement of a current generated by O_2 reduction [4]	-
pCO_2	Severinghouse principle – potentiometric measurement of pH change on the electrode, caused by CO_2 [4]	-
SO_2	Measurement of light absorbed by whole blood in 4 different wavelengths - sample is subjected to light radiation, while scattered light is also measured [4]	-
$cHCO_3^-$	Calculated [5]: $cHCO_3^- = 0.0307 \times PCO_2 \times 10^{(pH - 6.105)}$	-
BE	Calculated [5]: $BE = (1 - 0.014 \times tHb) \times [(1.43 \times tHb + 7.7) (pH - 7.4) - 24.8 + cHCO_3^-]$	-
$ctCO_2$	Calculated [5]: $ctCO_2(P) = cHCO_3^- + (0.0307 \times PCO_2)$	-
AG	Calculated [5]: $AG = Na^+ + K^+ - Cl^- - cHCO_3^-$	-

IFCC, International Federation of Clinical Chemistry and Laboratory Medicine [1] Labquest semi-automatic analyzer, Labtest Diagnostic, Lagoa Santa, MG, Brazil. [2] 9180 electrolyte analyzer, Roche Diagnostics GmbH, Mannheim, Germany. [3] Densitometer CS-9301PC, Shimadzu Corporation, Tokyo, Japan. [4] Roche OMNI C, Roche Diagnostics GmbH, Mannheim, Germany. [5] These variables were calculated directly by the Roche OMNI C analyzer.

Table 2. Age and body weight data of 28 buffalo calves.

		Male (*n*=16)			Female (*n*=12)			Total (*n*=28)		
	SI Units	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max
Age	Days	18.6	5.52	10-29	19.0	6.63	10-30	18.8	5.91	10-30
Weight	Kg	51.5	12.1	35.8-80.4	52.8	14.2	31.8-81.7	52.1	12.8	31.8-81.7

Table 3. Serum biochemical RIs for buffalo calves 10-30 days old, analyzed using Labquest semi-automatic analyzer (Labtest Diagnostic, Lagoa Santa, MG, Brazil) and 9180 electrolyte analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

		Descriptive Statistics				RI with 90% CI		Reference Value Advisor	
Analyte	SI Units	Mean	SD	Median	Min-Max	Lower Limit (90% CI)	Upper Limit (90% CI)	*n*	Method
GLU	mmol/L	4.76	1.02	4.84	2.86-6.60	2.65 (2.17-3.25)	6.99 (6.44-7.44)	28	BCTRD
DB	µmol/L	6.84	3.42	6.84	2.39-19.84	1.71 (1.71-3.42)	17.1(11.97-22.23)	28	BCTRD
TB	µmol/L	18.81	10.26	18.81	5.30-51.98	5.13 (3.42-6.84)	49.59(35.91-63.27)	28	BCTRD
AST	IU/L	105	28.9	102	52.4-178	53.5 (44.2-64.5)	174 (151-194)	28	BCTRD
ALP	IU/L	188	94.3	168	58.0-431	44.6 (35.1-65.8)	427 (346-501)	28	BCTRD
GGT	IU/L	64.9	82.1	30.6	11.5-348	10.3 (8.20-13.6)	598 (179-717)	28	BCTSD
CK	IU/L	251	176	210	76.5-999	93.2 (78.8-114)	914 (498-1910)	28	BCTRD
LDH	IU/L	1034	292	947	559-1676	552 (484-641)	1821 (1475-2085)	28	BCTRD
CREA	µmol/L	123.76	17.68	123.76	97.24-155.58	88.4 (79.56-106.08)	159.12(150.28-167.96)	28	BCTRD
Urea	mmol/L	4.06	1.67	3.78	2.00-8.26	1.83 (1.67-2.15)	8.57 (6.78-10.71)	28	BCTRD
CHOL	mmol/L	2.16	0.53	2.10	1.28-3.13	1.22 (1.08-1.39)	3.42 (3.00-3.81)	28	BCTRD
TG	mmol/L	0.22	0.09	0.20	0.09-0.42	0.08 (0.07-0.10)	0.44 (0.36-0.52)	28	BCTRD
Ca	mmol/L	2.52	0.25	2.47	2.19-3.12	1.97 (1.85-2.07)	3.02 (2.82-3.14)	28	URD
P	mmol/L	2.65	0.42	2.49	2.03-3.75	1.84 (1.71-2.00)	3.52 (3.20-3.81)	28	BCTRD
Mg	mmol/L	0.99	0.12	0.99	0.76-1.22	0.74 (0.66-0.78)	1.28 (1.19-1.36)	28	BCTRD
Na	mmol/L	139	3.30	139	134-147	132 (130-134)	146 (144-148)	27	BCTRD
K	mmol/L	4.70	0.4	4.70	4.10-5.40	3.90 (3.80-4.10)	5.60 (5.30-5.70)	27	BCTRD
iCa	mmol/L	0.600	0.200	0.600	0.350-0.97	0.200 (0.100-0.300)	1.00 (0.900-1.10)	27	BCTSD
Cl	mmol/L	97.6	7.70	97.1	82.8-115	82.8 (79.9-86.2)	115 (110-120)	28	BCTRD
Iron	µg/dL	157	69.8	156	42.8-274	34.4 (16.3-55.0)	329 (285-378)	28	BCTRD
TP	g/L	74.0	11.0	75.0	47.4-93.8	49.0 (42.0-57.0)	95.0 (90.0-100)	28	BCTRD
ALB	g/L	29.0	4.00	29.0	22.0-43.0	22.0 (21.0-24.0)	38.0 (35.0-42.0)	28	BCTRD

n indicates the number of animals; URD, Untransformed robust data; BCTSD, Box–Cox transformed standard data; BCTRD, Box–Cox transformed robust data; Glucose (GLU); Creatinine (CREA); Cholesterol (CHOL); Triglycerides (TG); Total protein (TP); Albumin (ALB).

Table 4. Venous blood gas variables RIs for buffalo calves 10-30 days old, analyzed using Roche OMNI C (Roche Diagnostics GmbH, Mannheim, Germany).

		Descriptive Statistics				RI with 90% CI		Reference Value Advisor	
Analyte	Units	Mean	SD	Median	Min/Max	Lower Limit (90% CI)	Upper Limit (90% CI)	*n*	Method
pH	-	7.40	0	7.40	7.25/7.42	7.30 (7.30/7.30)	7.40 (7.40/7.40)	28	URD
pO_2	mmHg	36.1	5.50	37.1	19.3/46.8	23.0 (15.4/28.5)	45.9 (43.4/48.0)	28	BCTRD
pCO_2	mmHg	46.7	4.40	47.3	35.0/55.9	36.7 (32.8/40.0)	55.2 (52.9/57.0)	28	BCTRD
SO_2	%	57.8	6.40	57.4	47.2/69.8	45.9 (43.7/48.8)	73.4 (67.6/78.7)	23	BCTRD
$cHCO_3^-$	mmol/L	25.2	2.60	25.3	18.1/30.6	19.6 (17.4/21.4)	30.3 (28.9/31.6)	28	BCTRD
BE	mmol/L	-0.800	2.40	-0.700	-6.90/4.20	-6.60 (-9.30/-4.50)	3.60 (2.50/4.50)	28	BCTRD
$ctCO_2$	mmol/L	22.5	2.20	22.4	16.1/27.3	17.5 (16.1/19.0)	26.6 (25.3/27.8)	28	BCTRD
AG	mmol/L	18.1	3.20	18.6	7.60/22.7	9.80 (4.50/13.10)	23.0 (22.0/23.8)	28	BCTRD

n indicates the number of animals; BCTRD, Box–Cox transformed robust data; URD, Untransformed robust data.