3 Materials and Methods

3.1 Experimental animals

The calves: The following experimental studies of the calves were made on an approved animal experiment (TV), with an annual renewal of the permit test (TV-Gene Approval number 0116/03). 32 clinically healthy calves, German Black-coloured and cross breeds (age: 4-104 days) were used to study acid-base status of the blood. The animals were examined clinically every 2 hrs during the experimental period of 8 hrs and after 24 hrs. The calves were assigned into 6 groups according to their age. The number and the age of the calves are presented in Table 7.

Table 7 Number and age of the calves during the experimental period (mean±SD).

Number of calves	Age	Age (days)
3	1st week of life	5 ± 1
6	2nd week of life	10 ± 2.3
5	3rd week of life	18.2 ± 2.2
4	4th week of life	27.5 ± 1.3
9	1 - 2 months	38.8 ± 6.9
5	2 - 3 months	79.6 ± 14.2

The young camels: 24 clinically healthy young camels (*Camelus dromedarius*) (age: ≤3-5 m) were used in this study. The young camels were assigned into 3 groups according to their age. The number and the age of the young camels are presented in Table 8. 14 healthy young camels of similar age and type and 22 adult camels (12 males and 10 females) were used to provide the control data (Table 8).

Table 8 Number and age of the experimental young camels and the control data.

Experimental young camels		Control data		
No	Age	No	Age	Sex
6	≤ 3 months	12	5-8 yrs	Adult males
8	3 - 4 months	10	5-8 yrs	Lactated females
10	4 - 5 months	14	3-5 months	Young camels (males and females)

3.2 Housing and management [1]

The study took place at the Clinic of Animal Reproduction and Institute of Veterinary Physiology, Faculty of Veterinary Medicine, Free University of Berlin, Germany and Department of Physiology and Camel Research Unit, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

The calves were housed individually in indoor pens and standard calf management procedures were used. The calves were fed milk which was provided three times daily (7:30 am, 13:30 and 22:30 pm) and the calves had free access to fresh water. Hay and grain were offered to the diet when the calves were 2 weeks old.

The young camels were housed in an outdoor environment in a shaded corral. The young camels were fed fresh grass (Abu sebean) and concentrates, which was provided daily. The young camels had free access to water.

3.3 Induction of the experimental metabolic acidosis

The experimentally metabolic acidosis was induced by using intravenous infusion of 5M NH_4Cl (pH = 5.05, Osmolality = 893 mOsmol/kg) at a dose of 1.0 ml/kg. The final solution was prepared by diluting the calculated dose of 5M NH_4Cl by 1:10 with distilled water to avoid any complications due to the higher osmolality of 5M NH_4Cl. The final solution was infused via a permanent intravenous catheter (14G x 50 mm, Vasofix® Braunüle (calves), 14G x 8 cm, Braunüle MT® (young camels), B/BRAUN, Germany) for a period of 2-2.5 hrs. The catheter was fixed to the skin by surgical suture. The animals were observed during the infusion by monitoring the respiratory and heart rate.

3.4 Clinical examination

The animals were examined every 2 hrs during the experimental period of 8 hrs, 24 and 48 hrs (for young camels) after the beginning of the intravenous infusion. Rectal temperature was monitored before the infusion by using a digital thermometer (Hans Dinslage D, Germany). Respiratory rate (breaths/min) was measured by auscultation of the thorax for a minimum of one minute using a clinical stethoscope. Heart rate (beats/min) was measured by auscultation of the thorax for a minimum of 30 seconds using a clinical stethoscope. The clinical

[1)] I would like to thank the workers at the Clinic of Animal Reproduction (Director: Univ Prof. Dr. W. Heuwieser) for providing the calves for the experimental work.

parameters were monitored every 2 hrs at the same time for blood collection and 24 and 48 hrs (for young camels) after the beginning of the intravenous infusion.

3.5 Samples collection

Blood collection: Blood samples were drawn every 2 hrs from the jugular vein via the permanent catheter by using heparinised plastic syringes (1.0 ml, Klinika Medical GmbH, Germany). The syringes were immediately sealed with a rubber cap and stored on crushed ice until analysed for venous blood pH, blood gases, and determination of various acid-base parameters and blood Hct, which were performed within 10 min of sampling. For the young camels, the venous blood pH was determined by using a pH meter (Labor Alliance, Germany). Camels' Hct (l/l) was determined by using a mircohaematocrit centrifuge. The initiation of the intravenous infusion of 5M NH_4Cl was designated as zero time. 7.5 ml non-heparinised syringes (Pirmvetta®, Laboratory Technique, GmbH, Germany) were used for the blood collection. The blood samples were centrifuged and the serum was collected in sterile containers for further analysis of serum electrolytes concentration, total protein, albumin, serum osmolality and creatinine.

Urine collection: The urine samples were collected in sterile containers via free catch or by perineal or preputial stimulation of the calves every 2 hrs at the same time of the blood collection and they were stored for further analysis.

3.6 Samples analysis

Blood gas analysis: The fresh blood samples from the calves were used for the determination of venous blood pH, P_{CO2}, blood- [HCO_3^-], blood- [BE] and Hct by using a blood gas analyser CCX (Nova biomedical GmbH-Adam-Opel., Rödermark, Germany). The values were corrected for the calves' rectal temperature.

Serum analysis: Automated biochemical analysis procedure conducted by the Institute of Veterinary Medicine Diagnostic Laboratory (IVD), Berlin, Germany were used for the determination of serum concentration of sodium (Na^+), potassium (K^+), chloride (Cl^-), inorganic phosphate (Pi), total protein, albumin and creatinine by using a biochemical analyser (Roche Hitachi Modular , Fa. Roche Diagnostics, Berlin, Germany) (Table 9a).

Serum osmolality: Serum samples were used for the determination of serum osmolality by using a freezing point-depression osmometer (Osmomat 030: D, gonotec, Germany) (Table 9a).

Table 9a Serum parameters and methodology of measurement

Parameter	Methodology	Equipment	CV (%)
Na^+	Ion selective electrode	Roche Hitachi Modular, Fa. Roche Diagnostics	1.26
K^+			1.77
Cl^-			2.39
Pi	Ammonium molybdate complex		2.71
Creatinine	Kinetic colour test after Jaffé		2.59
Osmolality	Freezing point-depression measurement	Osmomat. 030: D	1.3
Total protein	Serum protein capillary electrophoresis	Roche Hitachi 904 Roche Diagnostics	
Albumin			

CV: Coefficient of variation

3.7 SID_3 and $[A_{tot}]$ calculation

Serum concentrations of Na^+, K^+, Cl^-, Pi, and albumin were used for the calculation of serum-$[SID_3]$ and serum- $[A_{tot}]$ as described by Stewart, (1983), Constable et al., (2005) and Rehm et al., (2004).

$$[SID_3]\ (mmol/l) = ([Na^+] + [K^+] - [Cl^-])\ (mmol/l) \quad (1)$$

$$[A_{tot}]\ (mmol/l) = [Albumin]\ (g/l) \times 0.123\ (pH - 0.631) + [Pi]\ (mmol/l) \times 0.309\ (pH - 0.469) \quad (2)$$

3.8 Urine analysis

Urine pH: Fresh urine samples were used to determine urine pH by using a pH meter (InoLab, Scientific Technical Workshops, Weilheim, Germany) (Table 9b). The pH-meter was calibrated by using a two-point calibration with pH of 10.0-5.0. The pH probe was flushed with distilled water between measurements.

Urine osmolality: Fresh urine samples (calves) and stored urine samples (young camels) were used to determine urine osmolality by using a freezing point-depression osmometer (Osmomat. 030: D, gonotec, Germany) (Table 9b).

Urine electrolytes: Stored urine samples were used to determine the concentration of Na^+, K^+, Cl^-, Pi and creatinine in urine by using a biochemical analyser (Roche Hitachi Modular, Fa. Roche Diagnostics, Berlin, Germany) (Table 9b).

Table 9b Urine parameters and methodology of measurement

Parameter	Methodology	Equipment	CV (%)
pH	Ion selective electrode	pH meter	
Osmolality	Freezing point-depression measurement	Osmomat. 030: D	1.3
Na^+	Ion selective electrode	Roche Hitachi Modular, Fa. Roche Diagnostics	2.53
K^+			3.2
Cl^-			
Pi	Ammonium molybdate complex		2.51
Creatinine	Kinetic colour test after Jaffé		1.92

CV: Coefficient of variation

Fractional excretion of electrolytes: Serum and urine electrolytes and creatinine concentrations were used for the calculation of fractional excretion of electrolytes as described by Fleming et al., (1991):

$$FE_{electrolyte}(\%) = \frac{Urine - [electrolyte]}{Plasma - [electrolyte]} \times \frac{Plasma - [creatinine]}{Urine - [creatinine]} \times 100 \qquad (3)$$

3.9 Statistical analysis [2]

Statistical analysis was performed by using SPSS for Windows version 12.0. One-sample T-test was used to estimate the relationship between parameters and time (T-test versus initial value at time = 0 hrs). ANOVA tests (Levine's Test and Post Hoc Test) were used to assess the significant difference between the age groups. Linear regression procedures were used to estimate the relationship between venous blood pH and acid-base parameters, and the relationship between serum- [SID_3] and serum- [electrolyte] and between serum- [A_{tot}], serum- [albumin] and serum- [Pi]. Sigma Plot for Windows version 8.0 was used for the calculation of the area under curve (AUC). AUC procedure was used to estimate the animals' response during the experimental period. AUC values expressed as median value. The results are given generally as graphs to illustrate any changes in the mean values over the time and to explain the relationship between venous blood pH and acid-base parameters. The shaded area in the graphs represents the normal values (Stöber and Gründer, 1990).

[2)] I would like to thank Mrs R. Schmitz and Mrs. Dr. G. Arndt, Institute of Biometry and Data processing for statistical assistant.