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Assessment of Acid-Base Status, Certain Serum Biochemical and Urine Parameters during the Neonatal Period in Dairy Calves

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Abstract

The objective of the study was to assess acid-base status, certain serum and urine parameters during the neonatal period in claves. Twenty clinically healthy neonate calves (German Blacked-coloured and cross breeds, aged: 4-28 days) were used. The neonates were monitored from birth up to 28 days of age. Jugular venous blood samples were collected to determine various acid-base and serum parameters. Blood gas analysis was performed for pH, PCO_2, HCO_3^- and base excess (BE). Serum samples were used to determine $[Na^+]$, $[K^+]$, $[Cl^-]$, [Pi], total proteins (TP), albumin and osmolality. Anion gap (AG), strong ion difference (SID_3), total plasma concentration of non-volatile weak acids (A_{tot}) and strong ion gap (SIG) were calculated using validated equations. Urine samples were used to determine urine pH, osmolality and urinary-$[Na^+]$, $[K^+]$, $[Cl^-]$, and for the calculation of urine SID_3. The mean values of pH, PCO_2, $[HCO_3^-]$, [BE], [AG], $[SID_3]$, $[A_{tot}]$ and [SIG] were 7.39 ±0.02, 6.4±0.9 kPa, 29.4±3.9, 4.2±3.9, 15.5±3, 45.2±2.9, 12.3±1.8 and 3.2±3.8 mmol/l, respectively. Serum- $[Na^+]$, $[K^+]$, $[Cl^-]$, [Pi], [TP], [albumin] and osmolality showed mean values of 139±2.8, 4.9±0.4, 98.7±2.4, 2.8±0.3 mmol/l, 59±7.6, 26±3 g/l, and 283±6.6 mOsmol/kg, whereas urine pH**,** osmolality, urinary- $[Na^+]$, $[K^+]$, $[Cl^-]$ and $[SID_3]$ were 6.7±0.5, 214±137 mOsmol/kg, 22±17, 47±33, 37±15 and 28±26 mmol/l, respectively. During the neonatal period, the pH correlated positively ($P<0.0001$) to $[SID_3]$ while PCO_2, $[HCO_3^-]$ and [BE] correlated ($P<0.0001$) positively to each other and negatively to [AG] and [SIG]. Serum-$[SID_3]$ correlated ($P<0.0001$) positively to the pH and [BE]. Serum-[AG] correlated ($P<0.0001$) positively to [SIG]. A positive correlation ($P<0.05$) was reported between $[Na^+]$-$[Cl^-]$, $[Na^+]$-[TP], $[Cl^-]$-[albumin], [Pi]-[albumin], whereas the correlation was negative between [albumin] and [osmolality] ($P<0.0001$). The urine pH correlated positively ($P<0.0001$) to urine-$[Na^+]$ while urine osmolality correlated ($P<0.0001$) positively to $[K^+]$, $[SID_3]$ and $[Cl^-]$ ($P<0.01$).

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A positive correlation ($P \leq 0.05$) was observed between urinary-[Na^+], [K^+], [Cl^-] and [SID_3]. The neonatal period has an influence on acid-base status, serum electrolytes, albumin, and total proteins concentration. The data can utilise for the clinical monitoring of metabolic and acid-base disorders associated with the neonatal period. The data can be useful to develop a new strategy for the improvement of neonate care.

Keywords: Acid-base status; calves; neonatal period; serum and urine parameters; reference range.

1. Introduction

The neonatal period is defined as the period from delivery until 28 days; is considered as one of the most critical periods in the development of the physiological functions [1, 2, 3]. The neonatal period is also considered as a dynamic state for the neonate animals due to the intrinsic adjustments to the extra-uterine environment [2]. Therefore, many morphological, physiological and behavioural changes take place during the neonatal period to ensure a successful adaptation to the extra-uterine environment [4]. In newborn calves [5] concluded that the most physiological changes observed during the neonatal period are attributed to the dynamic changes in the energy sources from a maternal nutrient supply (carbohydrates and amino acids) to a diet rich in fat (colostrum and milk). An earlier study conducted by [6] stated that the most important physiological adaptation was that the neonate animals must be able to maintain adequate oxygen saturation, regulate acid-base status, engage endogenous metabolic pathways for energy production, and maintain body temperature within the physiological limits. During the neonatal period, the neonate animals are predisposed to acid-base imbalances as a consequence result of uterine contraction and foetal membranes rupture that occurred during the parturition, which alters respiratory components of the acid-base balance and thus high morbidity and mortality rates [1, 7]. Many researchers stated that the incidence of several acid- base disorders reported during this period [8, 9, 10, 11]. The literature concerning the evaluation of acid-base parameters during the neonatal period varied compared to the values reported for calves more than 28 days [12, 3, 13]. Therefore, many investigators recommended the analysis of blood gases and metabolites and renal function for the assessment of acid-base homeostasis to monitor the health status of neonate's calves [1, 14]. The kidneys are well known as being the main organs in the body responsible for the regulation of the extracellular electrolyte concentration and acid-base status of mammals by adjusting urine electrolyte excretion to maintain constant systemic pH [15, 16]. In neonate calves, a close relationship has been observed between acid-base homeostasis and renal function [3, 17]. In human infants, [18] observed lower glomerular filtration rate due to hypo-renal perfusion accompanied by immature tubular transport mechanisms for H^+ and HCO_3^-. Furthermore, many investigators concluded that the renal function modified with advancing age to satisfy the demands of animal's growth or depend on their nutritional status [19, 20]. Many investigators studied acid-base homeostasis, certain blood metabolites and urine parameters during the neonatal period in calves [1], goat kids [21] and lambs [22]. Therefore, the study aimed to provide additional information about acid-base status during the neonatal period in calves, which can be useful for the clinical monitoring of health status, diagnosis, and treatment of neonatal diseases.

2. Materials and Methods

2.1. Ethical Approval

The following experimental studies were performed on an approved animal experiment (TV-Gene Approval number 0116/03).

2.2. Animals and management

Twenty healthy neonate calves (German Blacked coloured and cross breeds, aged: 4-28 days) were used. The neonates were housed in individually in indoor pens and the standard calf management procedures were used (Clinic of Ruminants, Faculty of Veterinary Medicine, Free University of Berlin, Germany). The calves were fed milk, which was provided three times daily (7:30 am, 13:30 and 22:30 pm). Hay and grain were added to the diet when the calves were 2 weeks old. The calves had free access to fresh water.

2.3. Measurement of clinical parameters

The rectal temperature, respiratory rate (RR) and heart rate (HR) were measured using standard clinical procedures [7].

2.4. Sample Collection and Laboratory Analysis

Jugular venous blood samples were collected via a permanent intravenous catheter using heparinised plastic syringes (1.0 and 7 ml, Klinika Medical GmbH, Germany). The syringes (1.0 ml) were immediately sealed with a rubber cap and stored on crushed ice for blood gas analysis. Serum samples were collected after centrifugation in sterile Eppendorf vials to determine certain serum parameters. The urine samples were collected in sterile containers via a free catch or by perineal or preputial stimulation at the same time for the blood collection.

2.5. Blood gas analysis

The blood samples were analysed within 10 min after sampling for the determination of blood pH, PCO_2, blood-[HCO_3^-] and [BE], and blood haematocrit (HCT) using a blood gas analyser CCX (Nova biomedical GmbH-Adam-Opel., Rödermark, Germany). The values were corrected for the calves' rectal temperature.

2.6. Serum and urine analysis

The serum and urine concentration of sodium (Na^+), potassium (K^+) and chloride (Cl^-) (Ion-selective electrode), and creatinine (Kinetic colour test after Jaffé) were determined using an automated biochemical analyser (Roche Hitachi Modular, Fa. Roche Diagnostics, Veterinary Medicine Diagnostic Laboratory (IVD), Berlin, Germany). Serum and urine osmolality was performed using a freezing point-depression osmometer (OSMOMAT 030, gonotec, Germany).

2.7. Urine pH and urine osmolality

Ten ml of fresh urine samples were used to determine urine pH using a pH meter (InoLab, Scientific Technical

Workshops, Weilheim, Germany). The pH-meter was calibrated using a two-point calibration with pH 10.0-5.0. The pH probe was flushed with distilled water between measurements. The rest urine samples were used to determine electrolytes and creatinine concentration and urine osmolality.

2.8. Calculation of acid-base parameters [AG], [SID3], [Atot] and [SIG]

Serum concentrations of HCO_3^-, Na^+, K^+, Cl^-, Pi, and albumin were used for the calculation of serum- [SID_3] and serum- [A_{tot}] using validated equations.

$$[AG]\ (mmol/l) = ([Na^+] - [Cl^-] - [HCO_3^-])\ (mmol/l) \quad (1)\ [23]$$

$$[SID_3]\ (mmol/l) = ([Na^+] + [K^+] - [Cl^-])\ (mmol/l) \quad (2)\ [24]$$

$$[A_{tot}]\ (mmol/l) = [Albumin]\ (g/l) \times 0.123\ (pH - 0.631) + [Pi]\ (mmol/l) \times 0.309\ (pH - 0.469) \quad (3)\ [25]$$

$$[SIG]\ (mmol/l) = \frac{[Atot]}{1+10^{(pka-pH)}} + AG \quad (4)\ [26]$$

2.9. Statistical analysis

Statistical analysis was performed using SPSS for Windows version 20.0. The normal distribution of the individual data was determined using a One-Sample Kolmogorov-Smirnov adjustment test. The statistical analysis of certain acid-base, serum and urine parameters was estimated using descriptive statistics procedures of the same programme. A correlation was performed to assess the relationship between the parameters investigated. The mean difference was considered significant at $P \leq 0.05$.

3. Results

3.1. Acid-base parameters

The statistical data of acid-base parameters is shown in Table 3.1. The mean values of pH, PCO_2, [HCO_3^-], [BE] and [AG] were 7.39 ±0.02 (Reference range: 7.32– 7.43), 6.4±0.9 kPa (Reference range: 5.4 – 8.9), 29.4±3.9 mmol/l (Reference range: 25– 42), 4.2±3.9 mmol/l (Reference range: -0.1–17) and 15.5±3 mmol/l (Reference range: 7.1–22), respectively. Serum-[SID_3], [A_{tot}] and [SIG] showed mean values of 45.2±2.9 mmol/l (Reference range: 39–51), 12.3±1.8 mmol/l (Reference range: 10–14) and 3.2±3.8 mmol/l (Reference range: -5 –11), respectively.

[1] Mean±SD ×1.96 indicated the lower and the upper limits, Brackets ([ ]) donate concentration

PCO_2: partial pressure of carbon dioxide, BE: base excess, SID: strong ion difference, A_{tot}: total concentration of non-volatile weak acids, SIG: strong ion gap, AG: anion gap

Table 3.1: Statistical data of acid-base parameters of the neonate calves (n= 20)

Statistical values	pH	P_{CO2} (kPa)	$[HCO_3^-]$ (mmol/l)	[BE] (mmol/l)	[AG] (mmol/l)	$[SID_3]$ (mmol/l)	$[A_{tot}]$ (mmol/l)	[SIG] (mmol/l)
Mean±SD	7.39 ±0.02	6.4 ±0.9	29.4 ±3.9	4.2 ±3.9	15.5 ±3.7	45.2 ±2.9	12.3 ±1.8	3.2 ±3.8
Reference range[1]	7.32 – 7.43	5.4 – 8.9	25 – 42	-0.1–17	7.1 – 22	39 –51	10 –14	-5 –11
Median (1.-3. Quartile)	7.395 (7.38 – 7.4)	6.2 (5.8 – 6.6)	28.6 (27– 31)	3.3 (1.1– 5.8)	16 (14 –18)	45 (43–47)	12.5 (12 – 13)	3.9 (1.3 – 5.7)

Table 3.2 shows that during the neonatal period, the pH correlated positively (P<0.0001) to $[SID_3]$ while PCO_2 correlated (P<0.0001) positively to $[HCO_3^-]$ and [BE], and negatively to [SIG] and [AG]. Serum- $[HCO_3^-]$ correlated (P<0.0001) positively to PCO_2 and [BE] and negatively to [SIG] and [AG], whereas [BE] correlated positively to PCO_2, $[HCO_3^-]$ (P<0.0001) and $[SID_3]$ (P<0.01), and negatively to [SIG] and [AG] (P<0.0001). Serum- $[SID_3]$ correlated (P<0.0001) positively to pH and [BE]. The [AG] and [SIG] correlated (P<0.0001) negatively to PCO_2, $[HCO_3^-]$ and [BE] while [AG] correlated (P<0.0001) positively to [SIG].

Table 3.2: Correlation coefficients between acid-base parameters of the neonate calves (n= 20)

Parameter	pH	PCO_2 (kPa)	$[HCO_3^-]$ (mmol/l)	[BE] (mmol/l)	$[SID_3]$ (mmol/l)	$[A_{tot}]$ (mmol/l)	[SIG] (mmol/l)	[AG] (mmol/l)
pH	1							
PCO_2 (kPa)	-0.249	1						
$[HCO_3^-]$ (mmol/l)	0.178	0.900**	1					
[BE] (mmol/l)	0.274	0.852**	0.994**	1				
$[SID_3]$ (mmol/l)	0.723**	0.083	0.448	0.523*	1			
$[A_{tot}]$ (mmol/l)	0.058	-0.082	-0.064	-0.067	-0.366	1		
[SIG] (mmol/l)	0.271	-0.830**	-0.692**	-0.636**	0.282	-0.359	1	
[AG] (mmol/l)	0.370	-0.906**	-0.738**	-0.675**	0.272	-0.082	0.958**	1

Correlation coefficient is significant at: * P≤0.05; **P≤0.01

3.2. Clinical and serum biochemical parameters

The results shown in Tables 3.3 and 3.4 indicated that the mean values of RR, HR and HCT were 46±19 (25–90) breath/min, 113±23 (76–146) beat/min and 0.26 (0.18–0.32) l/l, respectively. Serum-[Na^+], [K^+], [Cl^-], [Pi], [TP], [albumin] and osmolality showed reference ranges of 130–143 (139±2.8 mmol/l), 4.3–5.8 (4.9±0.4 mmol/l), 94–101(98.7±2.4 mmol/l), 2.3–3.4 (2.8±0.3 mmol/l), 42–73 (59±7.6 g/l), 20–31 (26±3 g/l), and 270–292 (283±6.6 mOsmol/kg), respectively.

Table 3.3: Statistical data of clinical parameters, Hct and osmolality of the neonate calves (n= 20)

Statistical values	RR (breath/min)	HR (beat/min)	Hct (l/l)	Osmolality (mOsmol/kg)
Mean±SD	46 ±19	113 ±23	0.26 ±0.03	283 ±6.6
Reference range[1]	25– 90	76 – 146	0.18 – 0.32	270 – 292
Median	48	113	0.27	287
(1.-3. Quartile)	28 – 58	94 – 138	0.23– 0.29	277 – 288

[1] Mean±SD ×1.96 indicated the lower and the upper limits

RR: respiratory rate, HR: Heart rate, Hct: blood haematocrit

Table 3.4: Statistical data of certain serum biochemical parameters of the neonate calves (n= 20)

Statistical values	[Na^+] (mmol/l)	[K^+] (mmol/l)	[Cl^-] (mmol/l)	[Pi] (mmol/l)	[TP] (g/l)	[albumin] (g/l)
Mean±SD	139 ±2.8	4.9 ±0.4	98.7 ±2.4	2.8 ±0.3	59 ±7.6	26 ±3.0
Reference range[1]	130 –143	4.3 – 5.8	94 –101	2.3 – 3.4	42 – 73	20 – 31
Median	140	4.9	99	2.8	58	26
(1.-3. Quartile)	138– 141	4.6 – 5.1	97 – 101	2.6 – 3	55 – 66	24 – 28

[1] Mean±SD ×1.96 indicated the lower and the upper limits

Brackets ([ ]) donate concentration, TP: total proteins . Table 3.5 shows that the serum-[Na^+] correlated positively ($P<0.05$) to [Cl^-] and [TP], whereas [Cl^-] correlated positively ($P<0.05$) to [Na^+] and [albumin]. Serum-[albumin] correlated negatively ($P<0.05$) to serum osmolality and positively to [Cl^-] ($P<0.0001$) and [Pi] ($P<0.05$).

Table 3.5: Correlation coefficients between certain serum biochemical parameters of the neonate calves (n= 20)

Parameter	Osmolality (mOsmol/kg)	[Na^+] (mmol/l)	[K^+] (mmol/l)	[Cl^-] (mmol/l)	[Pi] (mmol/l)	[TP] (g/l)	[albumin] (g/l)
Osmolality (mOsmol/kg)	1						
[Na^+] (mmol/l)	0.300	1					
[K^+] (mmol/l)	0.386	0.274	1				
[Cl^-] (mmol/l)	-0.291	0.471*	-0.368	1			
[Pi] (mmol/l)	-0.139	0.253	-0.143	0.374	1		
[TP] (g/l)	0.198	-0.496*	-0.374	-0.302	0.051	1	
[albumin] (g/l)	-0.467*	0.041	-0.334	0.652**	0.500*	-0.047	1

Correlation coefficient is significant at: * $P\leq0.05$; ** $P\leq0.01$

3.3. Urine parameters

The results shown in Table 3.6 indicated that the mean values of urine pH**,** urine osmolality, urine-[Na^+], [K^+], [Cl^-] and [SID_3] were 6.7±0.5 (range: 5.9–7.5), 214±137 mOsmol/kg (range: 66–427), 22±17 mmol/l (range: 7–57), 47±33 mmol/l (range: 13–101), 37±15 mmol/l (range: 15–80) and 28±26 mmol/l (range: 10–92), respectively. Table 3.7 indicates that the urine pH correlated positively ($P<0.0001$) to urine-[Na^+] while urine osmolality correlated positively to [K^+] ($P<0.0001$), [SID_3] and [Cl^-] ($P<0.01$). A significant positive correlation ($P\leq0.05$) been observed between urine-([Na^+], [Cl^-], [SID_3]) and ([K^+], [Cl^-], [SID_3]), and between ([Cl^-], [SID_3]).

Table 3.6: Statistical data of urine parameters of the neonate calves (n= 20)

Statistical values	pH	Osmolality (mOsmol/kg)	[Na^+] (mmol/l)	[K^+] (mmol/l)	[Cl^-] (mmol/l)	[SID_3] (mmol/l)
Mean±SD	6.7 ±0.5	214 ±137	22 ±17	47 ±33	37 ±15	28 ±26
Reference range[1]	5.9 – 7.5	66 – 427	7 – 57	13 – 101	15 – 80	10 – 92
Median	6.7	172	15	34	31	10
(1.-3. Quartile)	6.3 – 7.2	92 – 385	10 – 31	20 – 91	23 – 49	7 – 45

[1] Mean±SD ×1.96 indicated the lower and the upper limits, Brackets ([ ]) donate concentration

Table 3.7: Correlation coefficients between urine parameters of the neonate calves (n= 20)

Parameter	pH	Osmolality (mOsmol/kg)	[Na^+] (mmol/l)	[K^+] (mmol/l)	[Cl^-] (mmol/l)	[SID_3] (mmol/l)
Osmolality mOsmol/kg)	0.168	1				
[Na^+] (mmol/l)	0.621*	0.497	1			
[K^+] (mmol/l)	0.286	0.910**	0.475	1		
[Cl^-] (mmol/l)	0.234	0.656*	0.732**	0.711**	1	
[SID_3] (mmol/l)	0.493	0.852**	0.644*	0.898**	0.566*	1

Correlation coefficient is significant at: * P≤0.05; **P≤0.01

4. Discussion

The data obtained in the present study revealed that acid-base homeostasis, serum and urine parameters were modified during the neonatal period as a normal physiological adaptation to the extra-uterine environment, and to satisfy the growth requirement. The findings are supported by the significant changes in the parameters investigated. The results in agreement with the findings reported by [4] who observed many endocrine and metabolic adaptations to extra-uterine life in neonate calves. The results obtained in the present study indicated that the reference ranges of acid-base parameters (pH, PCO_2, [HCO_3^-], [BE], [AG] and [SID_3]) were within the reference range reported previously by [1]; however, the above mentioned values showed slight higher values than that reported by [10]. The variation between the values in the present study and those reported previously might be due to age-related difference and the nutritional status of the calves. Furthermore, the negative correlation between the respiratory components of acid-base balance (PCO_2, HCO_3^- and BE) and the metabolic components (serum-[SIG] and [AG]) reported in the present study could reflected that the changes in the respiratory control mechanism caused a decrease in unmeasured cations and anions (equation 1) as a physiological adjustment between the respiratory and metabolic components of acid-base balance. In the present study, the reference ranges of RR, HR and HCT obtained for the neonate calves were within the reference range reported previously by [6, 27] (30-60 breath/min, 100-140 beat/min and 0.21.0.3l/l, respectively). Although PCO_2 was markedly higher in the neonate calves, the RR was not change significantly and maintained with the normal range for calves. This pattern of response demonstrates that the efficiency of the lungs to eliminate CO_2 in the neonate calves depended mainly on the chemical control of the respiration via chemoreceptors, resulting in a higher oxygen uptake [6, 1] and thus normal liver and kidney functions as indicated by the normal values of total proteins and albumin, and urine parameters (Tables 3.4 and 3.6). The reference ranges of urine parameters (pH, Na^+, K^+, Cl^- and osmolality) obtained in the present study for the neonate's calves were within the reference range reported previously by [28, 1], which can be also explained the optimum internal environment for the normal physiological function to ensure the electrolytes homeostasis and thus normal osmolality. Moreover, the positive correlation between the main electrolytes (Na^+ and Cl^-, the main contributors to plasma and urine osmolality, Na^+, K^+, Cl^- and urine SID_3) reported being correlated positively. This pattern of electrolytes response could reflect the synergic role of body organs (lungs, kidneys and liver) to maintain electrolytes and

acid-base haemostasis during the neonatal period in calves. (Tables 3.3 and 3.4). On the other hand, the positive correlation between the urine pH and Na^+ could reflect also that the elimination of H^+ during the neonatal period depend mainly on the activity of Na^+/K^+ ATPase, Na^+/H^+ antiporter and lesser to $Na+/Cl^-$ cotransporter. Furthermore, [17] stated that urinary excretion of electrolytes is a complex process involving filtration, reabsorption and secretion in various areas of the nephron via certain plasma membrane transporters. Thus, many investigators reported that each of these processes influenced by the age [20, 29, 21], the status of body fluids and the activity of various hormones [17]. Therefore, the critical attention of this situation should be considered in fluid therapy of these animals.

5. Conclusions

The slight variations in the values of acid-base parameters obtained compared to those reported previously in the literature due to the age factor. The physiological adaptation to the extra-uterine environment and the nutritional status of the neonate's calves play a significant role in these variations.

6. Recommendations

The study recommends that the data can employ for the clinical monitoring of metabolic and acid-base disorders associated with the neonatal period. The data can be useful to develop a new strategy for the improvement of neonate care. Critical attention should be considered in the feeding programme and fluid therapy during the neonatal period.

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