5 Discussion

One of the main objectives of the present study is the induction of a non-respiratory (metabolic) acidosis in clinically healthy calves and young camels by using intravenous infusion of 5M NH_4Cl (Dilution 1:10, measured pH: 5.05, $[H^+]$: 8912.5 nmol/l, $[SID_3]$: -500 mmol/l, $[A_{tot}]$: 0 mmol/l and measured osmolality: 893 mOsmol/kg). The 5M NH_4Cl solution used was characterised by a high osmolality and thus bound water causing plasma haemodilution as evidenced by a decrease in the blood Hct in both calves and young camels (Fig. 4.3a and 4.24a). It is well known that the ECF occupies about 20% of BW (about 10000 ml/50 kg, 18000 ml/90 kg) and the plasma occupies 5% of BW (about 2.500 ml/50 kg, and 4.500 ml/90 kg). According to the used dose (1.0 ml/kg) for a calf weighing 50 kg (50 ml of NH_4Cl) and a young camel weighing 90 kg (90 ml of NH_4Cl), the haemodilution effect of this solution was low because the solution diffused faster into both the plasma (2%) and the ECF (0.5%). It seems that the present study is the first study to evaluate the response of acid-base regulatory mechanisms during the induction of metabolic acidosis for a period of 8 hrs and after 24 and 48 hrs. The present study is also the first documentation of SID theory in young camels with experimentally induced metabolic acidosis. The main finding of the present study is that intravenous infusion of 5M NH_4Cl has been successfully used to induce an experimental metabolic acidosis in calves and young camels with normal acid-base status (pH= 7.40±0.01 and 7.42±0.01, respectively). This is characterised by a transient decrease in venous blood pH (Figs. 4.11a and 4.32a), a decrease in venous blood- P_{CO2} (Fig. 4.12a), venous blood- $[HCO_3^-]$ (Fig. 4.13a), venous blood- $[BE]$ (Fig. 4.14a) and the significant decrease in measured serum- $[SID_3]$ (Fig. 4.16a and 4.33a). The effect appears to be time dependent and a moderate metabolic acidosis (a hyperchloraemic acidosis) was observed by 4 hrs after the beginning of the infusion.

5.1 Response of clinical parameters to the experimentally metabolic acidosis

Respiratory rate (breaths/min): The results obtained in the present study indicate that metabolic acidosis was accompanied by a respiratory compensation in form of a moderate increase in RR in both calves and young camels (30-65 breaths/min and 13-20 breaths/min, respectively) and hypocapnia in calves (5.2-5.6 kPa, Fig. 4.12a). Hypocapnia is known to be stimulated by the action of H^+ on the central and peripheral chemoreceptors (Robinson, 2002). This pattern of respiratory compensation to the experimental metabolic acidosis is consistent with previous studies in diarrhoeic calves with severe metabolic acidosis (Naylor, 1987; Berchtold et al., 2000; Omole et al., 2001). In contrast, Hartmann et al., (1997) reported that

almost two third of 34 diarrhoeic calves showed metabolic acidosis with no respiratory compensation due to severe acidosis and hypovolaemia. The same authors observed that one quarter of the calves had a mix respiratory-metabolic acidosis.

Heart rate (beats/min): The present results show a noticeable significant decrease in HR after 4 hrs, which is remained at a lower level during the experimental period until 6 hrs in calves and after 48 hrs in young camels (80-115 beats/min, calves and 41-44 beats/min, young camels, Figs. 4.2a and 4.23a). This could be related to the observed hyperkalaemia in calves (4.9-5.9 mmol/l, Fig. 4.5a). However, the changes in HR in young camels may be due to the hyponatraemia observed (146-148 mmol/l, Fig. 4.25a). Previous studies in calves concluded that metabolic acidosis contributes to cause cardiac rhythm abnormalities by decreasing myocardial potassium, elevating extracellular potassium and changing membrane potential (Weldon et al., 1992). As serum- $[K^+]$ increases (>7 mmol/l) the resting membrane potential drops, shortening the duration of the action potential, and eventually decreasing the excitability of the atrial myocyte. In addition, the atrial myocardium may become refectory (Weldon et al., 1992). Previous studies in diarrhoeic calves have shown that metabolic acidosis associated with hyperkalaemia lead to cardiac rate or rhythm abnormalities characterised by bradycardia (Weldon et al., 1992).

5.2   Response of blood Hct to the experimentally metabolic acidosis

The results obtained in the present study show that intravenous infusion of 5M NH_4Cl resulted in a decrease in blood Hct (0.24-0.26 l/l, calves and 0.25-0.26 l/l, young camels, Figs. 4.3a and 4.24a). This might be due the higher osmolality of the NH_4Cl solution used (893 mOsmol/kg) which leads to a compensatory movement of the fluids from ICF to ECF. However, the absence of the significant change in the blood Hct in calves indicates that there were no major changes in the ECF volume. The more pronounced decrease in the blood Hct in young camels might be due attributable to the accumulative effect of serum hyperosmolality due to the infusion above the normal higher serum osmolality (normal osmolality of young camels = 304 mOsmol/kg). The results suggest that the slight decrease in the Hct in calves and the significant decrease in the Hct in young camels could reflect intracellular volume contraction which might be an additional stimulus to aldosterone release. Previous studies of rats demonstrated that acute metabolic acidosis by using NH_4Cl has been known to increase aldosterone level in rat kidney (Wang et al., 1998). Recent study conducted by Iwabushi et al., (2003) concluded that induction of metabolic acidosis by using 5M NH_4Cl caused a significant decrease in blood Hct in calves.

5.3   Response of serum parameters to the experimentally metabolic acidosis

Serum- [Na$^+$]: Na$^+$ is the principal extracellular ion that is used as an indication of body water and it is an important ion responsible for the regulation of osmotic pressure, acid-base balance and maintenance of membrane potentials (DiBartola, 1998). In the present study, the young camels seem to have higher concentrations of serum Na$^+$ and K$^+$ (Figs. 4.25a and 4.26a) than those for calves (Figs. 4.4a and 4.5a). This may reflect species-dependent differences in water uptake. Normally, camels only drink small amounts of water, which may lead to higher concentration levels of serum ions.

The results obtained in the present study show that intravenous infusion of a solution free sodium (5M NH$_4$Cl) associated with a significant trend towards hyponatraemia in the older animals (141 mmol/l, calves and 141-146 mmol/l, young camels, Figs. 4.4a and 4.25a). Hyponatraemia has been observed previously in association with metabolic acidosis in diarrhoeic calves (Lorenz et al., 1998; Grove-White and Michell, 2001). This pattern of response of serum- [Na$^+$] to the experimentally induced metabolic acidosis reflects a possible autoregulation that observes 2 hrs after the beginning of the intravenous infusion.

In young animals, there was a distinct decrease in serum- [Na$^+$] by 2 hrs after the beginning of the intravenous infusion, and the serum- [Na$^+$] did not change remarkably with time in all age groups. Omole et al., (2001) concluded that metabolic acidosis has no significant influence on serum- [Na$^+$] in diarrhoeic calves.

Serum- [K$^+$]: As mentioned, the serum biochemical profile obtained in the present study show a marked hyperkalaemia after 2 hrs in all calves (4.9-5.9 mmol/l, Fig. 4.5a). Surprisingly, in the young camels with experimentally induced metabolic acidosis, hypokalaemia was observed after 2-8 hrs (4.2-4.6 mmol/l, Fig. 4.26a). Possibly, this hypokalaemia can be attributed to the hypernatraemia observed (145-147 mmol/l, Fig. 4.25a). Similar results have been observed previously in diarrhoeic calves (Grove-White and Michell, 2001). On the other hand, metabolic acidosis has been observed to cause significant transcellular electrolyte shift whereby K$^+$ leaves the cell to the ECF in exchange for H$^+$ to maintain electroneutrality (Carlson, 1997; DiBartola, 1992). The hyperkalaemia observed after 2 hrs can be due to the exchange of extracellular K$^+$ for intracellular H$^+$ in response to the experimentally induced metabolic acidosis. Previously hyperkalaemia was a common problem associated with acid-base and electrolyte disturbances in neonatal calves with diarrhoea (Grove-White and Michell, 2001; Berchtold et al., 2000).

Serum- [Cl⁻]: The results reported in the present study indicate that intravenous infusion of $5M$ NH_4Cl in calves and young camels caused hyperchloraemia, which was observed by 2 hrs after the beginning of the infusion. In the calves, the more pronounced hyperchloraemia observed after 4 hrs was accompanied by a pronounced decrease in the plasma concentration of HCO_3^-, reflecting an exchange for Cl^- to maintain electroneutrality. Constable, (2000) reported that an excessive administration of Cl^- containing fluid resulted in hyperchloraemia. The results also indicated that the serum- [Cl⁻] increased gradually with time in both calves (100-107 mmol/l) and young camels (114-115 mmol/l) (Fig. 4.5a and 4.27a) presumably because of the inverse relationship between serum- [Cl⁻] and serum- [HCO_3^-]. Many researchers concluded that the increase in serum- [Cl⁻] compensates for the decrease in serum- [HCO_3^-] (Hartmann et al., 1997; Grove-White and Michell, 2001). This has been confirmed in the present study and hyperchloraemia (Fig. 4.5b) was associated with hypobicarbontaemia in calves (21-26 mmol/l, Fig. 4.13a) and a marked decrease in venous blood pH in both calves and young camels (7.32-7.36 and 7.32-7.34, respectively, Fig. 4.11a and 4.32a). Furthermore, hyperchloraemia can cause a decrease in systemic pH by depressing the reabsorption of HCO_3^- in the proximal renal tubules. HCO_3^- normally moves along with Na^+ into the tubular cells and thus to the blood (DiBartola, 1992).

Serum- [Pi]: The results obtained in the present study show a significant hypophosphataemia after 6 hrs in calves and after 8 hrs in young camels (Figs. 4.7b and 4.28b). The changes in the serum- [Pi] probably reflect the autoregulation mechanisms that happen in response to the experimentally induced metabolic acidosis. In contrast, previous studies conducted by Walker et al., (1998) concluded that experimentally induced osmotic diarrhoea was associated with hyperphosphataemia in young calves.

Serum- [Protein]: The role of plasma proteins in acid-base balance is well recognised in humans (Stämpfli and Constable, 2003) and animals (Constable, 2000). The present results show that both calves and young camels responded to the intravenous infusion of the protein free solution (5M NH_4Cl) by decreasing serum- [Protein]. However, the young camels showed the more pronounced significant decrease in serum- [Protein] after 4 hrs (48-51.2 g/l, Fig. 4.29a) compared with the slight decrease that occurred in calves. In the young camels, the present results demonstrate the importance of plasma proteins in acid-base balance in addition to the regulation that happens normally by serum electrolytes (SID).

Serum- [Albumin]: In the present study the initial mean values of serum- [Albumin] for the youngest calves in the 1ˢᵗ week of life are lower compared with the reference range reported by Stöber and Gründer, (1990). This may be due to an increase in serum- [globulin] observed

(Appendix A$_3$). These results are in agreement with those reported previously by Knowles et al., (2000).

The serum- [Albumin] of the calves and young camels had a pattern similar to that of the serum- [Protein] and normalised more rapidly by 24 hrs after the beginning of the infusion, possibly correcting with a normalisation of blood pH (about 7.40). In calves, the changes in serum- [Albumin] delayed until 8 hrs, suggesting that the autoregulatory mechanisms of acid-base balance by the liver are slow in comparison with the respiratory and the renal regulatory mechanisms. The general pattern of the young camels' response indicated that the changes in serum- [Albumin] to the intravenous infusion were more pronounced than that of the calves' response. This may be due to the age-difference between the calves (1st w-3 m) and the young camels ($\leq$3-5 m). Generally, the significant decrease in serum- [Albumin] during the experimental period explains the role of serum albumin as an extracellular buffer. The results also indicate that the decrease in serum- [albumin] may cause a marked decrease in serum- [A$_{tot}$] and consequently affect acid-base status.

Serum osmolality: Under physiological conditions, plasma osmolality is regulated by changes in water balance, which are in turn determined by thirst, enteric absorption and renal excretion of water (Standon and Koeppen, 1990; DiBartola, 1992). Therefore, an excretion of urine with an osmolality different from that of plasma will result in a marked change in plasma osmolality. In newborn calves until 10 days, the normal plasma osmolality is about 280 mOsmol/kg (Bulter et al., 1971). Other investigators reported a value of 298 mOsmol/kg in diarrhoeic calves before death (Fisher and De La Fuente, 1972). The results reported in the present study show that the initial mean values of serum osmolality were between 276-289 mOsmol/kg which can be considered as normal. The control data presented in Table 10a show that the normal serum osmolality of the camels was higher (301-308 mOsmol/kg) compared with the calves (276-289 mOsmol/kg). This may due to the presence of higher serum- [Na$^+$] in the camels (150 mmol/l) compared with those of calves (138-143 mmol/l).

In response to the intravenous infusion of 5M NH$_4$Cl (osmolality = 893 mOsmol/kg) there was a gradual significant increase in the serum osmolality with time in both calves and young camels (281-294 mOsmol/kg, calves and 300-308 mOsmol/kg, young camels, Figs. 4.10a and 4.31a), which in turn stimulated the secretion of ADH. This pattern of response is similar to that observed in calves with experimentally induced metabolic acidosis using 5M NH$_4$Cl (Iwabushi et al., 2003). The present results also show that the highest hyperosmolality was observed by 8 hrs after of the beginning of the infusion, suggesting that the response of the plasma osmolality regulatory mechanisms to the higher osmolality of 5M NH$_4$Cl reached the

peak after 8 hrs. The decrease in the serum osmolality occurred after 24 and 48 hrs explains that the autoregulatory mechanisms were continuously elevated in order to regain the normal osmolality. This pattern of serum osmolality response accompanied by significant changes in urine pH and urine osmolality observed (Figs. 4.19, 4.36, 4.20 and 4.37, respectively).

In young camels, serum osmolality fell below the initial values (Fig. 4.37a) with differences in the time of the appearance. The decrease in serum osmolality observed accompanied by a significant decrease in Hct (Fig. 4.24a), indicating the autoregulation of water balance following haemodilution of ECF due to the higher osmolality of 5M NH_4Cl (893 mOsmol/kg). It is well known that Na^+ is responsible for 90% of the serum osmatically effective cations in the plasma and in the whole ECF. Therefore, any changes in serum- $[Na^+]$ will directly affect serum osmolality. This is confirmed in the present results and hyponatraemia observed after 48 hrs (141-146 mmol/l, Fig. 4.25a) in young camels was associated with a significant decrease in the serum osmolality (295-304 mOsmol/kg, Fig. 4.31a).

5.4   Response of acid-base parameters to the experimentally metabolic acidosis

Henderson- Hasselbalch variables

Blood pH: The results obtained in the present study show a rapid and significant decrease in blood pH by 2 hrs after the beginning of the infusion in both calves and young camels (7.32-7.36, calves, 7.32-7.34, young camels, Figs. 4.11a and 4.32a). A sustained gradual decrease in blood pH with time was also persisted throughout the experimental period until 8 hrs. This effect is probably due to the direct adjustment in the ratio of $[HCO_3^-]/ [H_2CO_3]$ buffer and thus of blood pH in accordance with the Henderson and Hasselbalch equation (Carlson, 1997). Also it may be due to the decrease in serum SID (Stewart, 1983). The significant decrease in pH reported in the present study is in agreement with that reported previously in calves with experimentally induced metabolic acidosis using 5M NH_4Cl (Iwabushi et al., 2003) and in diarrhoeic calves (Berchtold et al., 2000; Grove White and White, 2001; Ewaschuk et al., 2004).

Blood P_{CO2}: Acid-base variables such as P_{CO2}, HCO_3^- and BE demonstrate the benefits in this study. The present results indicate that there was a wide variation in the initial values of venous blood P_{CO2} in all age groups. This can be explained by the individual variations between the calves. In response to the experimentally induced metabolic acidosis, venous blood P_{CO2} decreased linearly with time (5.2-5.6 kPa, Fig. 4.12a), indicating that there was a respiratory compensation for the changes in blood pH. In the present study, the marked

changes in venous blood P_{CO_2} in the young calves during the first 4 weeks of life did not attain statistical significance (Fig. 4.12b). This suggests that the respiratory compensation for metabolic acidosis is poor or slow in the young calves. This is consistent with previous studies of diarrhoeic calves (Kasari and Naylor, 1986; Naylor, 1987). In young calves, it seems that the respiratory compensation for metabolic acidosis was impaired, probably due to immature lung function, mainly gas exchange function. Studies of humans have shown that the maturation of the alveoli and its gas exchange function increase with age from infants (23 days) to adulthood (Zeltner et al., 1987). Furthermore, Burri, (2006) concluded that the alveolar formation in rats occurred within a very short period of about 10 days. The same author observed that microvascular maturation in humans started very early (3 days) and continued until the age of 2-3 years.

The results obtained in the present study showed that the venous blood P_{CO_2} remained at a lower level after 24 hrs in the old calves (1-2 m). This can be explained by the continuous respiratory compensation to the experimental metabolic acidosis. Previous study conducted by Naylor, (1987) concluded that the diarrhoeic calves showed a respiratory compensation for at least 24 hrs after the persistence of metabolic acidosis.

Blood- [HCO$_3^-$]: The general pattern of serum HCO_3^- (Fig. 4.13a) obtained in the present study likely explains the blood pH response. Based on the Henderson-Hasselbalch theory, the alterations in the blood- [HCO_3^-] or blood P_{CO_2} have an immediate effect on blood pH which has been observed in the present study (Fig. 4.15b). According to DiBartola, (1992) there is a reciprocal increase in serum chloride to balance the decrease in blood HCO_3^-. This has been also confirmed in the present study and hypobicarbontaemia observed (Fig. 4.13a) was accompanied by hyperchloraemia (Fig. 4.6a). The significant decrease in blood- [HCO_3^-] observed in the present study is in agreement with previous studies in healthy calves with experimentally induced metabolic acidosis (Iwabushi et al., 2003) and in diarrhoeic calves (Kasari and Naylor, 1986; Weldon et al., 1992; Suzuki et al., 2002).

Blood- [BE]: The results reported in the present study show that the youngest calves in the 1st week of life had higher blood- [BE] values than the older ones. This has also been observed in a previous study conducted by Naylor and Forsyth, (1986). They concluded that young calves of 2-5 days old had BE values that exceeded those of the calves of 11-18 days.

The results obtained in the present study show that blood- [BE] decreased in response to the intravenous infusion of 5M NH$_4$Cl to -0.3 mmol/l in all calves. This can be considered as a successful sign of the persistence of metabolic acidosis. Gentile et al., (2004) defined

metabolic acidosis by the decrease in blood- [BE] to < -3.0 mmol/l in healthy calves with experimentally induced ruminal acidosis. Moreover, Walker et al., (1998) reported a decrease in BE in healthy calves with experimentally induced osmotic diarrhoea. The same authors concluded that the decrease in BE maintained at positive values until 48 hrs. This has been also observed in the present study, particularly in the youngest calves in the 1st week of life and the blood- [BE] maintained at a positive value until 24 hrs. The decrease in the blood- [BE] reported in the present study is consistent with that reported previously in diarrhoeic calves (Berchtold et al., 2000; Omole et al., 2001; Lorenz, 2004).

According to the Henderson-Hasselbalch theory, the changes in pH can occur due to the changes in blood P_{CO2}, blood- [HCO_3^-] and serum- [BE]. The results obtained in the present study show that there was a significant change in the blood pH in response to the changes in the blood P_{CO2}, but this correlation was not strong ($R^2 = 0.01\text{-}0.02$). Therefore, the changes in blood pH did not correspond to the changes in P_{CO2} (Fig. 4.15a). This may be due to the fluctuations on the blood P_{CO2} during the experimental period which is not sufficient to make a marked influence on blood pH (Fig. 4.12a). In the young calves, the absence of the significant correlation between blood pH and P_{CO2} during the first 3 weeks of life (Fig. 4.15a) could related to the procedure for the measurement of P_{CO2} in venous blood instead of arterial blood which has been more accurate than the venous blood.

Conversely, the results obtained in the present study demonstrate a good correlation between blood pH and blood- [HCO_3^-] in all calves ($R^2 = 0.29\text{-}0.52$, P<0.01) (Fig. 4.15b). The present results indicate that the regulation of blood pH through HCO_3^- buffer is more effective in the old calves than that in young calves. These results suggest immature pulmonary and renal functions in young calves because HCO_3^- buffer is controlled normally by both the respiratory and the renal system.

The present results indicate that there was a marked age influence and the age of the calves (1st w-3 m) was consistent with calves' response compared with the young camels (age: ≤3-5 m). Moreover, the young calves until the 4th week of life were more acidotic than the older calves (Fig. 4.11b). This pattern of response is in agreement with previous studies in diarrhoeic calves (Grove-White and Michell, (2001) who reported that the younger calves more than 6 days old were more severely acidotic than the older ones. In contrast, Grove-White and White, (1993) concluded that the older calves tending to be more severe acidotic than the younger calves. While, Naylor, (1987) concluded that the diarrhoeic calves more than 8 days were more acidotic than the younger calves less than 8 days.

Stewart's variables

P_{CO_2} in calves: The effect of the experimentally induced metabolic acidosis on P_{CO_2} was discussed previously in the Henderson- Hasselbalch variables section.

Strong ion difference in calves: In calves, characterisation of acid-base disturbances by using strong ion difference approach has been established previously (Table 1). The normal serum SID_3 for healthy calves has been considered to be 43±2.4 mmol/l (Constable et al., 2005). However, the serum SID_3 reported in the present study (44.2-47.3 mmol/l, Fig. 4.16a) is higher than those reported previously by Constable et al., (2005) (Table 1) and lower than those reported by Grove-White and Michell, (2001) (Table 1). This may be due to the decrease in serum- $[Cl^-]$ observed (95-97.5 mmol/l, Fig. 4.5a) from the normal values reported by Stöber and Gründer, (1990). According to the Stewart's theory, metabolic acidosis is apparent as a decrease in SID. This has been confirmed in the present study and the decrease in serum- $[SID_3]$ (38-44 mmol/l) caused a marked decrease in systemic pH (7.32-7.36, Fig. 4.18a). The decrease in the venous blood pH was compensated by hyperventilation (30-65 breaths/min, Fig. 4.1a) and consequently caused a decrease in P_{CO_2} (5.2-5.6 kPa, Fig. 4.12a).

It is clear from equation (1): $SID_3 = [Na^+] + [K^+] - [Cl^-]$ that the changes in serum- $[SID_3]$ depended on the changes in one or more of the three strong ions: Na^+, K^+ and Cl^-. The present results indicate that the significant decreases in serum SID_3 could be related mainly to hyperchloraemia induced by infusion of NH_4Cl (Fig. 4.16c).

Strong ion difference in young camels: This study represents the first attempt to apply SID theory in camels. It is also the first description of the changes in metabolic components of clinically healthy young camels with experimentally induced metabolic acidosis by using intravenous infusion of 5M NH_4Cl (pH = 5.05, $[SID_3]$ = -500 mmol/l, $[A_{tot}]$ = 0 mmol/l and measured osmolality = 893 mOsmol/kg). Furthermore, it is also difficult to find reports about acid-base disorders in camels. Till now there is no published data is available regarding the normal values of serum- $[SID_3]$. The present study demonstrates that the SID_3 in camels is similar to those reported previously for calves and the other animals. The initial serum- $[SID_3]$ values (42-45 mmol/l, Fig. 4.33a) for the young camels are similar to the SID_3 values reported for the control data (43.4±2.2-46.6±3.7 mmol/l, Table 10b) and to those reported for the calves in the present study (44.2-47.3 mmol/l, Fig. 4.16a). They are also similar to those reported previously for calves (Constable et al., 2005) but different from those reported previously for horses (Stämpfli et al., 1999).

It is clear from the present study that there is a close relationship between serum- $[SID_3]$ and the three strong ions (Na^+, K^+ and Cl^-). The significant changes in these strong ions caused a marked change in serum- $[SID_3]$ (Figs. 4.33c, d and e). By 24 hrs after of the beginning of the infusion, the serum- $[SID_3]$ remained significantly lower than the initial values, suggesting that the acid-base regulatory mechanisms were incomplete and that the readjustment of normal acid-base balance is slow. As mentioned above the moderate hyperchloraemia appeared to be the main factor responsible for lowering SID_3.

The results obtained in the present study also indicate that the age and metabolic body weight had no influence on the camels' response. While, there was a marked age influence on the calves' response. This might be due to the age difference between the examined calves (1st w-3 m) compared with the young camels ($\leq$ 3-5 m). The current results suggest that age and metabolic body weight may have an influence on the response of the young camels (1st w-3 m).

A_{tot} in calves: In the present study, the initial serum- $[A_{tot}]$ values (12-13 mmol/l, Fig. 4.17a) for the calves are similar to those reported previously for calves (Constable et al., 2005). However, the serum- $[A_{tot}]$ is different from those reported for cattle (Constable, 2002), humans (Stämpfli and Constable, 2003) and horses (Stämpfli et al., 1999). In the present study, the decrease in serum SID_3 accompanied by a decrease in serum- $[A_{tot}]$, which can be considered as a compensatory metabolic alkalosis.

Plasma protein provides the major contribution to A_{tot} and therefore plasma proteins independently affect acid-base balance (Stämpfli and Constable, 2003). Stewart, (1983) proposed that A_{tot} is derived mathematically from measured values of albumin and Pi which albumin the main contributor to A_{tot}. Previous investigations in humans and calves have confirmed this assumption (Fencl et al., 2000; Constable et al., 2005). This is confirmed in the present study and the decrease in serum A_{tot} observed is determined mostly by the significant decrease in serum- [Albumin] and serum- [Pi] observed (Figs. 4.17c and 4.17d, respectively).

A_{tot} in young camels: In the present study, the initial serum- $[A_{tot}]$ values (12-13 mmol/l, Fig. 4.34a) for the young camels are similar to the control data (12-14 mmol/l, Table 10b) and to those of the calves (12-13 mmol/l, Fig. 4.17a). The analysis of the present results also indicates that the initial serum- $[A_{tot}]$ is similar to those reported previously for calves (Constable et al., 2005). However, the initial serum- $[A_{tot}]$ is different from those reported for humans (Constable et al., 1999) and horses (Stämpfli et al., 1999). The significant decrease in serum- $[A_{tot}]$ in the young camels appears to be due to the marked decrease in serum-

[albumin] observed (Fig. 4.34c) in addition to the significant changes in serum- [Pi] (Fig. 4.34d).

According to the chemical components of the protein free solution (5M NH_4Cl), it is clear that A_{tot} of this solution is 0 mmol/l and the drop in serum- [A_{tot}] might appear to be due to haemodilution effect as indicated by the drop in Hct. However, the delay in the drop suggests that the autoregulatory mechanisms in response to the presence of metabolic acidosis are more likely explanation.

The linear regression obtained in the present study for the calves show that the decrease in serum- [SID_3] observed associated with a significant decrease in venous blood pH in accordance with Stewart's theory ($R^2 = 0.80$, $P<0.01$, Fig. 4.18a). In young camels too, the results obtained in the present study are consistent with the Stewart theory (Figs. 4.35a and 4.35b, respectively).

5.6   Response of urine parameters to the experimentally metabolic acidosis

The present study demonstrates for the first time that experimentally induced metabolic acidosis by using 5M NH_4Cl in both calves and young camels associated with marked changes in the renal responses. This is characterised by the significant decrease in the urine pH, a significant increase in the urine osmolality and marked changes in the fractional excretion of electrolytes.

Urine pH: It is well established that the kidneys respond to the changes in the acid-base status by altering acid excretion (Koeppen et al., 1985). When the blood pH falls, the kidneys usually excrete urine with a pH of less than 5.5. Therefore, the acidic urine pH (5.5, calves and 6.2, young camels) observed during the experimental period of 8 hrs, after 24 and 48 hrs (Figs. 4.19 and 4.36) can be considered as an indicator of the renal response to the decrease in blood pH observed (Figs. 4.11a and 4.32a). Possibly, this is related to the increase in the activity of Na^+-H^+ antiporter and H^+-ATPase pump in response to the experimentally induced metabolic acidosis. Prasad et al., (1988) concluded that the brush border membrane of the rat renal proximal tubules responded to metabolic acidosis by increasing the activity of Na^+-H^+ antiporter. The significant decrease in the urine pH observed in the present study is in consistent with that reported by previous investigators in diarrhoeic calves (Ulutas and Sahal, 2005) and in adult cows fed a mixture of NH_4Cl and $MgSO_4$ (Wang and Beede, 1992; Löffler, 2004). Moreover, NH_4Cl is known to decrease urinary pH in dogs (Schober, 1996), cats (Funaba et al., 2001) and rats (Questin et al., 2004).

The results obtained in the present study clearly indicate the renal compensatory mechanisms response to the acute metabolic acidosis. The persistence of low pH after 24 and 48 hrs could be explained by the continuous renal excretion of H^+ in order to regain the normal acid-base status. In young animals (calves and camels), the significant changes in the urine pH were only observed 8 hrs after of the beginning of the infusion. This pattern of renal response might be due to immature renal function, in particular transport processes involved in electrolyte transport and the acid-base transport protein function of the kidneys. Previous studies of young rats (3 days old) have shown that there were a few proximal tubules in the renal cortex with few acid-base transporters mainly Na^+-H^+ antitransporter and Na^+-HCO_3^- cotransporter (Bonnici and Wagner, 2004). Therefore, the present study suggests that the delay in the renal response to metabolic acidosis in young animals, reflects immature Na^+-H^+ antiporter and H^+-ATPase pump which are responsible for H^+ secretion.

Urine osmolality: On the basis of the urine data, the results reported in the present study, show that the significant decrease in urine pH (Figs. 4.19 and 4.36) in both calves and young camels in response to experimentally induced metabolic acidosis associated with a significant increase in the urine osmolality (400-700 mOsmol/kg, calves and 1200-1400 mOsmol/kg, young camels, Figs. 4.20 and 4.37). This could be related to the increase in the secretion of ADH in response to serum hyperosmolality observed (Figs. 4.10a and 4.31a). An increase in urine osmolality has been observed in previous studies in rats with experimentally metabolic acidosis (Amlal et al., 2004). The same authors showed that NH_4Cl increased osmotic diuresis as a result of decreased salts and fluid reabsorption. In the young camels, the lower values of serum osmolality accompanied by a low excretion of Na^+ after 48 hrs compared with the initial values (Fig. 4.38a). This pattern of renal response indicating a close relationship between plasma osmolality and the renal concentrating mechanisms, and that the renal concentrating mechanisms are supported by the decrease in the urine osmolality occurred (Fig. 4.37). Recent studies demonstrated that chronic metabolic acidosis caused by NH_4Cl induced a significant increase in urine osmolality with no change in urinary Na^+ excretion in rats (Amlal et al., 2004). Conversely, in other studies metabolic acidosis was shown to decrease water and NaCl reabsorption in the proximal renal tubules (Wang et al., 1998). Therefore, the significant increase in the urine osmolality after 24 hrs in both calves and young camels could be related mainly to the induction of the experimentally metabolic acidosis using 5M NH_4Cl.

Fractional excretion of electrolytes (FE): Fractional excretion of electrolytes is calculated by comparing the amount of the substance excreted in the final urine with the amount filtrated

through the glomerulus. Na^+ and Pi are freely filtrated through the glomerulus and reabsorbed by the renal tubules. Therefore, the FE of Na^+ and Pi are indices of the tubular function. FE of electrolytes is a simple, inexpensive method that has been shown to be a reasonable indicator for renal clearance of electrolytes in calves (Sommardahl et al., 1997) and adult cattle (Fleming et al., 1992). However, there are a few reference data regarding the normal values of the FE of electrolytes in young calves. The initial mean values for FE $_{Na+}$, FE $_{K+}$, FE $_{Cl-}$ and FE $_{Pi}$ reported in the present study for the calves are similar to those reported for healthy calves (Sommardahl et al., 1997; Ulutas et al., 2005) and non-lactating cows (Fleming et al., 1992). However, the results differ from those reported previously for cows during, pre and post partum periods (Ulutas et al., 2003). The results also indicate that the age of the calves had a significant impact on the FE of electrolytes except for Pi. As the age of the calves increased the diet changed from all liquid (milk) to partially liquid. Therefore, the changes in the urinary fractional excretion of electrolytes are probably related mainly to the increase in water conservation and partially due to the decrease in water intake. On the other hand, the older calves showed higher values for FE $_{electrolyte}$ compared to the younger ones, suggesting immature renal function in the young animals as mentioned previously (Urine pH section). Also in the old calves the FE $_{Pi}$ was higher compared with the younger ones (Fig. 4.21d). This might be due to the increase in phosphate intake associated with an increase in ingestion of grain in the older calves.

In camels, there is no published data to demonstrate the evaluation of renal function and to estimate electrolyte concentrations in urine and for the calculation of renal FE $_{electrolyte.}$ Therefore, the results reported in the present study are the first documentation for the renal functions in camels. The main findings obtained in the present study show that in young camels the FE $_{Na+}$, FE $_{K+}$ and FE $_{Pi}$ were low, whereas FE $_{Cl-}$ was higher compared with that of calves. This can be explained by the morphological differences between the young camels and the calves' kidneys. Camels always try to maintain high levels of serum Na^+ and Cl^- concentrations by decreasing their excretion in urine.

The significant increase in the FE $_{K+}$ and FE $_{Cl-}$ during the experimental period and after 24 hrs in both calves and young camels can be considered as the main signs of the induction of the experimental metabolic acidosis. The noticeable increase in the FE $_{Na+}$ obtained in the present study in both calves and young camels (0.2-2%, calves and 0.05-0.3% young camels, Figs. 4.21a and 4.38a) accompanied by a significant increase in the FE $_{K+}$ (25-50%, calves and 20-35%, young camels, Figs. 4.21b and 4.38b), suggests an increase in aldosterone secretion in response to the experimentally induced metabolic acidosis. Previous studies of

rats have shown that metabolic acidosis can increase the excretion of Na^+, K^+ and Cl^- (Graber et al., 1981). The present results confirm these observations and the experimentally induced metabolic acidosis caused a marked increase in the FE_{Na+}, FE_{K+} and FE_{Cl-}. In contrast, Ulutes and Sahal, (2005) reported a decrease in FE_{Na+} and FE_{K+} with no changes in FE_{Cl-} in diarrhoeic calves. The increase in the FE_{Na+}, FE_{K+} and FE_{Cl-} can be explained by the role of the kidney to maintain serum concentration of these cations and anions along the nephron by increasing their secretion in urine. The more pronounced significant increase in the FE_{Na+}, FE_{K+} and FE_{Cl-} in response to the experimentally induced metabolic acidosis could be consider as a result of the renal compensation to the hyponatraemia, hyperkalaemia and hyperchloraemia observed (Figs. 4.25a, 4.5a, 4.6a and 4.27a, respectively). The significant increase in the FE_{Pi} observed in the young camels (0.8-2.0%, Fig. 4.38d) could related to the decrease in the activity of Na^+-Pi cotransporter in the renal proximal tubules in response to the experimentally induced metabolic acidosis. Pervious study of rats have concluded that metabolic acidosis has been observed in associated with a decreased activity of Na^+-Pi cotransporter (Prasad et al., 1988). The significant changes in the renal response to the experimentally induced metabolic acidosis might be due to the increase in the activity of the transport proteins such as Na^+-H^+ antiporter and Na^+-Pi cotransporter in the proximal tubules, an increase in the activity of Na^+-H^+ antiporter, H^+-ATPase pump in the distal tubes and the collecting ducts. In addition to the decrease in the activity of Na^+-K^+-2Cl symporter in the thick loop of Henle.

5.7   General conclusions

(1) Intravenous infusion of 5M NH_4Cl was successfully used to induce an experimental metabolic acidosis in both calves (1st week-3 m) and young camels ($\leq$3-5 m).

(2) Experimentally induced metabolic acidosis caused a significant increase in the respiratory rate (breaths/min) accompanied by a significant decrease in the heart rate (beats/min) after 4 hrs in both calves and young camels. The changes in the respiratory rate were age-dependent, whereas the age had no significant effect on the changes in the heart rate.

(3) Hyponatraemia was observed only in the old animals. The age had no significant effect on the animals' response to experimentally induced metabolic acidosis.

(4) In calves, hyperkalaemia was observed after 2 hrs and remained elevated for 24 hrs. The changes in serum- [K^+] in the young calves until the 4th week of life were more clearly compared with the older ones. Hypokalaemia was observed in the young camels after 2-6 hrs. The age had no significant effect on the camels' response.

(5) In both calves and young camels, hyperchloraemia was observed after 2 hrs and remained elevated for 24 hrs. The change in serum- [Cl$^-$] in response to the experimentally induced metabolic acidosis was age-dependent in calves. The age had no significant effect on the camels' response.

(6) In both calves and young camels, hypophosphataemia was observed after 2 hrs and remained elevated for 24 hrs. The age had a significant effect on the animals' response.

(7) Hypoproteinaemia and hypoalbuminaemia were observed only in the young camels. The age had a marked influence on the camels' response, whereas it had no significant effect on the calves' response.

(8) Blood Hct decreased significantly in response to the experimentally induced metabolic acidosis only in young camels. The age had no significant effect on the animals' response.

(9) Serum osmolality increased gradually with time until 24 hrs in all calves and in the old camels. The age had no significant effect on the animals' response.

(10) Venous blood pH decreased sharply in response to the experimentally induced metabolic acidosis in both calves and young camels. The young animals were more sensitive to the experimentally induced metabolic acidosis than the older ones.

(11) Venous blood P_{CO2}, blood- [HCO$_3^-$] and blood- [BE] decreased significantly in response to the experimentally induced metabolic acidosis in calves.

(12) Venous blood P_{CO2}, blood- [HCO$_3^-$] and blood- [BE] correlated to the venous blood pH in calves. Serum- [SID$_3$] and serum- [A$_{tot}$] had a marked influence on the venous blood pH in both calves and young camels.

(13) In calves, a reverse relationship was observed between venous blood pH, P_{CO2}, blood- [HCO$_3^-$], blood- [BE] serum- [SID$_3$] and serum- [A$_{tot}$] and metabolic body weight. The reverse relationship was observed in the young camels only for venous blood pH, serum- [SID$_3$] and serum- [A$_{tot}$].

(14) In both calves and young camels, serum- [SID$_3$] and serum- [A$_{tot}$] decreased significantly in response to the experimentally induced metabolic acidosis. The changes in serum- [SID$_3$] depended on the age and metabolic body weight in calves, whereas they had no effect in on serum- [SID$_3$] in young camels. Neither the age nor metabolic body weight had significant effects on the changes in serum- [A$_{tot}$] in both calves and young camels.

(15) Urine pH decreased gradually with time in response to the experimentally induced metabolic acidosis in both calves and young camels. Urine osmolality increased significantly by 24 hrs after the beginning of the infusion in both calves and young camels.

(16) FE_{Na+} increased in response to the intravenous infusion only in young camels. FE_{K+} and FE_{Cl-} increased in both calves and young camels. FE_{Pi} did not change significantly in response to the experimentally induced metabolic acidosis in calves, whereas it increased significantly in young camels.

(17) On the basis of our findings we concluded that there is a biologically significant difference between the experimental animals in relation to their age. It is clear that the young animals until the 4th week of life (calves) and ≤3 months (young camels) were more sensitive to induction of metabolic acidosis than the older ones.

(18) The results clearly explain a particular susceptibility of young animals to the neonatal diseases, in particular those leading to metabolic acidosis. Therefore, we recommended that a particular care must be taken to prevent the young animals.