The effect of a 48 hour fast on acid- base balance and blood gas values on dairy cows

Research Project Veterinary Medicine University Utrecht
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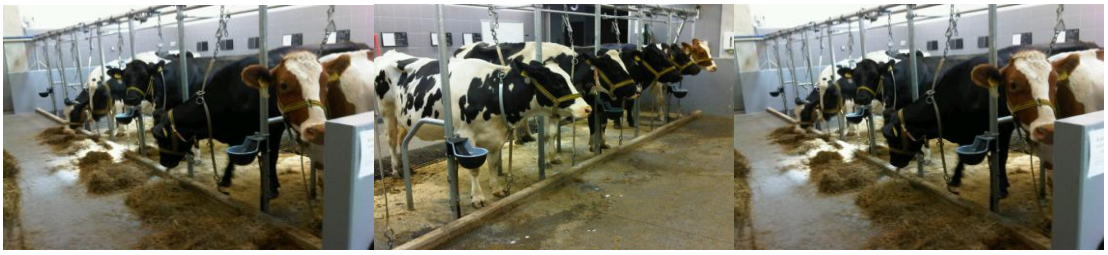


Prefatory note During the study of Veterinary medicine at the University of Utrecht, students have to fulfill a research project. This paper is the report of the research project of the master student A.E. Kist, at the University of Utrecht.
The research was done to know more about changes in acid-base balances and blood gas values in healthy lactating cows, during a 48 hour fast. The experiment took place at the education stalls at the Veterinary University of Utrecht.

Contents

Abstract The aim of this study was to study which changes were caused in the acid-base balance and electrolytes during fasting. Five healthy lactating cows were selected for the longitudinal study to be monitored over five and a half day. This period was divided into a non fasting period and a fasting period. During the first day the cows could get used to the situation and acclimatize to the environment, on the second day baseline measurements were performed, day three and day four were the fasting period and day five and six were the follow up.
Due to the fast, significant decreases were seen in pH and bicarbonate. A significant increase was seen in the total protein concentration. Changes in the electrolyte concentrations were seen but not all of them were significant. The conclusion of this study is that due to the unexpected dehydration a metabolic acidosis occurred. Electrolyte concentrations in plasma remain constant, probably due to the simultaneous occurrence of fasting and dehydration.

Introduction Energy intake is very important for lactating cows. Depriving dairy cows of food often leads to an imbalance of electrolytes, severe ketosis, increased concentrations of fatty acids and a decreased blood glucose concentration (Royce J et al., 1976). Literature about experiments of effects of fasting in dairy cows is restricted, almost all research is done in bulls and the outcomes are conflicting with each other. Therefore, in this study the cows are monitored before, during and after the fasting period. It is described that fasting influences electrolyte concentrations and acid-base balances in the extracellular fluid. The disturbed acid-base balance, due to the fast, is partly a result of a lack in electrolytes (Burton et al., 2001 and Galyean et al., 1981).
According to Stewarts approach, the acid-base balance can be influenced by three mechanisms; namely the strong ion difference (SID), the partial pressure of carbon dioxide (CO_2) (pCO_2) and the concentration of nonvolatile weak acids (Atot). Electrolytes play an important role in the first mechanism, namely; the SID is determined by the difference of the strong anions and cations (Constable et al., 2000).
Potassium is an important electrolyte in this study. A decreased intake of food and a decreased potassium intake due to the fast, results in a hypokalemia, because the kidneys need 24 - 48 hour to adjust to the situation (Sattler et al., 1998, Peek et al., 2003,). As a result, hypokalemia can cause a metabolic alkalosis by the exchange of intracellular K^+ into the blood and extracellular H^+ into the cells, so extracellular H^+ concentrations decrease (Peek et al.,2000).
During the fast, the passage through the abomasum is slowed down. The acid abomasal content contains high amounts of chloride ions and in the duodenum extracellular bicarbonate is exchanged with chloride, this is stimulated by the passage of a food bolus (Murphy et al., 2007). Due to the delayed passage, extracellular bicarbonate accumulates by the lack of stimulation and the decreased exchange with chloride. The accumulated bicarbonate enhances the metabolic alkalosis of the hypokalemia which is mentioned before (Story et al., 2006).
Another result of fasting is that the extracellular glucose concentrations decrease. Due to the decreased ruminal fill and activity of microbes, less glucose can be produced by gluconeogenesis. Glucose absorption across the gastrointestinal tract is also decreased. The ruminant blood glucose concentration is normally maintained at 60 – 80 mg / 100 ml, and can tolerate levels as low as 40 mg / 100 ml (Reece et al., 1993).
To maintain energy sources, two major actions take place during fasting; mobilization of glucose sources and utilization of other fuels, like mobilization of fatty acids, to spare glucose for metabolic mechanisms which are dependent on glucose. Products of these mechanisms are ketone bodies. Ketone bodies are weak acids and they can cause a metabolic acidosis (Saarinena et al., 1950). The expectation is that this acidosis is not strong enough to overcome the metabolic alkalosis, arised from the hypokalemia and delayed passage. These mechanisms are coördinated by hormones, mainly glucagon. Glucagon is produced within the alfa cells of the pancreatic islets, secretion is stimulated when blood glucose is low. The sensing mechanism

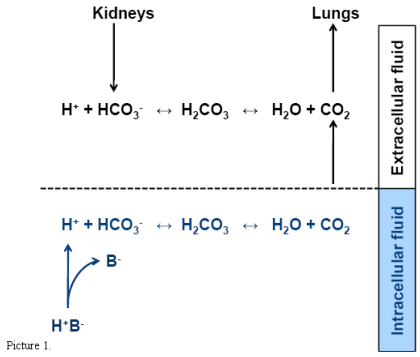
resides in the interior of the alfa cell. Insulin is necessary to transport glucose into the alfa cell. High levels of fatty acids inhibit the secretion of glucagon (Reece et al., 2004).
This study is used to study the effects of fasting on the acid-base balance and electrolytes in dairy cows.

Acid / Base balance The acid-base balance is a complicated mechanism, it involves many systems in the body.
Like other components of the extracellular fluid, the hydrogen (H^+) concentration is maintained within narrow limits.
Under normal circumstances, references of blood pH of a dairy cow are between 7,4 and 8,1 (Reece at al., 1993). The pH of a solution is defined as log 1 / $[H^+]$. The H^+ concentration which is responsible for the pH is low, consequently the pH is easily disturbed by addition of strong acids or bases (Constable et al., 2009).
The relative constant H^+ concentration in the extracellular fluid is the result of the acid-base balance mechanisms. Acids are substances that donate H^+ to the solution, bases are substances that accept H^+ from a solution and bind them. When acids or bases are added or removed, this balance shifts and becomes disturbed (Burton et al., 2001).
Hydrogen and bicarbonate ions are formed by the metabolism. Small amounts are formed from the oxidation of amino acids and the anaerobic metabolism of glucose. Large amounts are produced by oxidative aerobic metabolism, with the major source of acid; carbon dioxide as a result. CO_2 rapidly reacts with H_2O to form H_2CO_3, which dissociates into H^+ and bicarbonate (HCO_3^-) (Reece et al., 2004).
An increase in the acid concentration, leads to a decrease in pH and is called an academia. A decrease in the acid concentration, which leads to an increase in pH causes an alkalemia. Differences in pH can be caused by respiratory or metabolic components. Primary differences in pCO_2 levels in the blood are called a respiratory alkalosis or acidosis, primary differences in HCO_3^- are called a metabolic alkalosis or acidosis (Gorman et al., 1973).
As any other animal, cows require a balanced acid-base balance to survive and to keep their milk production at a high level. Regulators are blood, cells and lungs. Also intake of minerals has an impact on this balance (Kerndt et al., 1982). Minerals used in dietary cation-anion differences, named DCAD, are potassium sodium and chloride (Tremblay et al., 2006).
DCAD measuring levels of these four minerals, the cations; potassium and sodium, and the anions; chloride and sulfur are merged and the ratio DCAD is determined as $((Na^+K)-(Cl+S))$.
DCAD affects blood buffering capacity and extracellular acidity (Afzaal et al., 2004).
The acid-base balance is also affected by the passage of the abomasal acid content through the gastrointestinal tract. Normally chloride is exchanged for bicarbonate in the duodenum to neutralize the content of the duodenum. But if the passage is reduced or delayed, such as in a situation of fasting, it may be that this exchange is also reduced or delayed, so bicarbonate accumulates in the extracellular fluid, and chloride remains in the abomasal content. This results in a metabolic alkalosis (Murphy et al., 2007).

Buffers Buffers are necessary to keep the pH between narrow levels, a buffer system consists of a mixture of weak acids and their conjugate bases.
When a buffer is presented in the extracellular fluid, the addition of an acid or base results in a smaller pH shift compared to a situation where no buffer is present (Reece et al., 1993).
For example, when a strong acid is added to a solution, the H^+ ions are directly bound to the buffer bases to form weak acids.
The buffers are located in the extracellular- and intracellular fluids and in the bone (Fencl et al., 2000).
The three buffers which are discussed in this study are;

- Chemical buffers
- Respiratory buffers
- Renal buffers

Chemical Buffers; Chemical buffers neutralize the extracellular fluid directly to prevent changes in the acid-base balance.



Picture 1.

The most important extracellular buffer is bicarbonate; the HCO_3-/pCO_2 buffer, the components of this system are regulated by kidneys and lungs. The primary intracellular buffers are organic and inorganic phosphates and hemoglobin in the erythrocytes.

At a normal pH level, the hemoglobin buffer molecules are presented as proteinate ions. The base Hb- forms a pair with the weak acid HHb, when acid is added to the blood the reaction that follows is: H^+ + Hb- ←→HHb.

Plasma proteins also exist to a certain extent as buffer protein ions at body pH and bind H^+ in a similar way as hemoglobin does (Gorman et al.,1973).

The phosphate buffer: HPO_4/H_2PO_4 plays an important role in the kidneys and in the intracellular fluid, and a minor role in the extracellular fluid.

Bone also represents an important site of buffering. Bone buffering occurs by the exchange of excess extracellular H^+ with bone surface Na^+ and K^+(Reece at al., 1997).

Respiratory Buffers; Respiratory buffers react within minutes in a situation of an acidosis, to lose CO_2 by breathing.

The respiratory buffer system depends on the exquisite sensitivity of the control systems to changes in the extracellular CO_2 and pH (Reece et al., 1997).

Chemosensitive areas are very sensitive for changes in the H^+ concentration in the interstitial fluid of the brain. They are located near by the ventral surface of the medulla. There is also a peripheral chemoreceptor located at the bifurcation of the carotid arterie and at the arch of the aorta, named the carotid and aortic bodies. These receptors detect the changes in the partial pressures of carbon dioxide, oxygen and hydrogen ion concentrations and affect the respiratory center. The respiratory center is affected by impulses of the nerves from the carotid bodies; the glossopharyngeal nerves and nerves from the aortic arch; the nervus vagus. The center in the medulla is excitatory to the respiratory center, by causing an increase in volume and frequency.

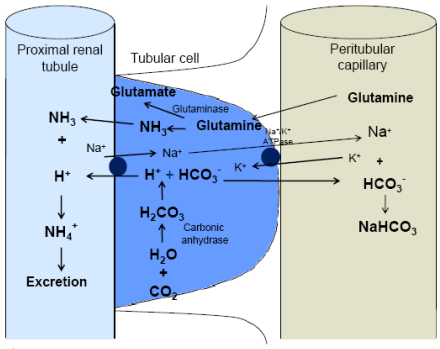
Blood pCO_2 can vary extensively, because of the buffer capacity of bicarbonate and because of the partial pressure of CO_2 in the lung alveoli which determines the amount of CO_2 dissolved in the blood.

A small decrease in pH and increase in blood CO_2 stimulates pulmonary ventilation, so CO_2 is washed out by an increase in expiration. A decreased extracellular CO_2 causes a decrease in H_2CO_3 concentration and so a decrease in blood pH.

When the pH decreases, the first reaction is the HCO_3^- buffer resulting in the formation of additional CO_2 and depletion of HCO_3^-. The increase of CO_2 and decrease of pH stimulates breathing, causing a rapid expiration of the CO_2. When the pH is still below normal, slow expiration of additional CO_2 occurs, because in that way arterial pCO_2 will decrease over a period of hours to a normal level.

In this way, the acid-base ratio is brought back between normal levels, whereas the buffer concentrations are not yet at their normal levels. So this compensatory mechanism needs further mechanisms, like renal buffers (Burton et al., 2001).

Renal Buffers; Renal buffers react within hours to days to correct the acid-base balance by the excretion and reabsorption of acids or bases (Burton et al., 2001).
In case of an acidosis, the excess H^+ can be excreted in urine, and HCO_3^- is formed in the tubules cells. In case of an alkalosis, the excess HCO_3^- can be excreted.
Changes in electrolyte concentrations in extracellular fluid are also mainly corrected by tubular reabsorption and excretion.
When the acid-base balance is disturbed, H^+ or HCO_3^- are excreted or reabsorbed to balance the extracellular pH.
Secretion and reabsorption of H^+ and HCO_3^- take place at all parts of the tubules, except the ascending and descending part of the lis of Henle (Reece et al., 1994).
In case of an extracellular acidosis, the excess H^+ ions are excreted in the urine. Filtered HCO_3^- will be completely reabsorbed.
Active secretion of H^+ takes place in the distal tubule and in the collecting tubes.
The quantity of acid that can be excreted as free H^+ ions is minimal so the urine needs a base buffer. The buffers which can bind H^+ in urine are phosphate and ammonium (Reece et al., 2004).
The phosphate(HPO_4) buffer is freely filtered in the glomerulus and binds with H^+ in the tubule to form H_2PO_4. The H^+ ions are excreted in exchange for sodium, NaH_2PO_4.
Under normal circumstances, 75 % of the filtered phosphate is reabsorbed, but in case of a strong acidosis there is not enough phosphate to buffer the urine so the ammonium buffer is stimulated.
The ammonium buffer is capable to buffer more H^+ than phosphate does.
The ammonium pathway starts in the liver with the protein metabolism, with NH_4 as a product. NH_4 is normally used to form urea, but in case of an acidosis, this is not useful because with the urea reaction an extra H^+ is formed. So, NH_4 is used to form glutamine. The production of glutamine is stimulated by an acidosis.



Picture 2.

As shown in picture 2, ammonia is formed from glutamine in the tubular cell, deamination of glutamine under the influence of glutaminase results in 2 NH_4 and alfa-ketoglutarate. NH_4 is excreted in tubular lumen, due to the anion trap, the ion cannot be reabsorbed and is excreted in the urine. Another product of the reaction is alfa- ketoglutarate, this product is oxidized into 2 HCO_3^- ions and co-transported with Na^+ back into the extracellular fluid (Afzaal et al., 2004 and Clarkson et al., 2005).
Every H^+ that is been bound by another buffer than HCO_3^-, a new HCO_3^- is formed by the tubular cells (Barton et al., 2001).
Kidneys are also able to regulate the extracellular fluid volume of the cow. Sodium is important to maintain this volume, due to the salt balance. When there is a decrease in the extracellular fluid volume, the anti diuretic hormone (ADH) and the thirst mechanism are stimulated, the animals start to drink and the extracellular volume increases. When the extracellular volume increases, sodium with water can be excreted. Excretion of plasma sodium involves first glomerular filtration and then tubular reabsorption (Burton et al., 2001). Changes in excretion can be generated from increases in plasma concentrations or glomerular filtration rate. The excretion starts very fast, but the reabsorption takes a little longer. Both H^+ and K^+ are exchanged for Na^+. An increase in H^+ concentration depresses K^+ secretion.
When the extracellular Na^+ concentration is low, a decrease in osmolarity is detected, inhibiting the ADH release and the urine volume increases. Aldosterone enhances sodium reabsorption

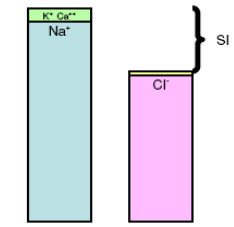
in the renal tubule and the secretion of potassium from tubular cells into the lumen. ACTH has a positive influence on aldosterone release. ACTH is released after stress and by increased levels of ADH in plasma (Reece et al., 2004).

Stewarts Approach In this experiment Stewarts Approach is used to determine the difference between the strong ion concentrations. This approach is useful when total protein, albumin and / or phosphates concentrations are disturbed.

In Stewarts approach is the acid-base balance determined by three independent variables; the strong ion difference (SID) which is mediated by the kidneys, the partial pressure (pCO_2) which is mediated by the lungs and the nonvolatile weak acids (Atot). The variables that depend on the independent variables are the pH and bicarbonate (Constable at al., 2008).

Atot is the sum of the dissociated and non-dissociated parts of the weak acids. The degree of dissociation is related to the pH or temperature. The main component of Atot are the plasma proteins (>90) and inorganic phosphates. Atot is determined by; [Atot] = [A'] + [HA] (Muir et al., 1994).

The SID is the difference between the fully dissociated strong anions and cations. Strong ions are those which not participate in proton transfer reactions and so dissociate completely in water, they determine the concentration of H^+ and OH^-, and so the pH. Strong cations are Na^+, K^+, Mg^{++} and Ca^{++}, strong anions are Cl^-, $BHBA^-$ and lactate, each applies a direct electrochemical and osmotic effect (Storyl et al., 2004). The ions which are used in the calculations are not altered by any of the reactions in the system, and none of the ions are produced or consumed (Fencl et al., 2000). The ion concentrations are imposed on the solution from the outside and are also controlled by outside mechanisms. Anions like Na^+ and Cl^- are inorganic strong ions, these ions are mainly absorbed from the intestinal. Control of these ions occur by variations in renal excretion and hormones due to the control system. Lactate and béta hydroxybutyraat acid (BHBA) are organic strong ions, produced by metabolic reactions and may be metabolized in the liver. The SID is determined by: SID = {[Na^+] + [K^+] + [Ca^{++}]} - {[Cl^-] + [lactate$^-$]} (Constable et al., 1999).



Picture 3.

As shown in picture 3, the excess of positive charge is the difference of the strong ions, this value is always positive because of the amount of cations and is balanced by an equal amount of buffers, like bicarbonate, albumin and phosphates.

An increase in SID indicates that OH^- is in excess of H^+ indicating an alkalosis, as a result of a contraction alkalosis, decrease of extracellular volume; resulting in increased sodium, or a loss of a strong ions, like chloride.

A decrease in SID indicates that H^+ is in excess of OH^- indicating an acidosis, due to a dilutional acidosis; an increase of extracellular volume combined with a decrease of Na^+ concentration, or an increase in anions (Fencl et al., 2000).

The anion gap (AG) is used for the differential diagnose of a metabolic acid-base problem. The anion gap is calculated with: AG= (Na^++K^+) - (Cl^-+HCO_3^-), and is usually between 12 – 16 mmol/L (Bradford et al., 2009). The positive AG value is due to the unmeasured anions, the measured kations are in the majority of the anions. The difference of the amount is a result of the fraction of unmeasured anions like serum proteins and other unmeasured anions like sulfate, phosphate and organic acids like lactate.

A change in the anion gap gives an indication of the cause of the change in the acid-base balance.

Decreases in the AG are rare, but increases are often seen. An increase in the AG value is due to an increase in cation concentrations. For example a decrease in the HCO_3^- concentration due to the buffer capacity resulting in an increased Cl^- concentration to compensate the

difference (Fencl et al., 2000). In a situation of fasting, anions like acetoacetate and lactate are produced, this is also leading to a decrease in HCO_3^- concentration because of the consuming buffer capacity and so in an increased AG (Story et al., 2006 and Reece et al., 1994).

Electrolytes In this experiment, potassium (K^+), chloride (Cl^-), sodium (Na^+) and calcium (Ca^{++}) are measured.

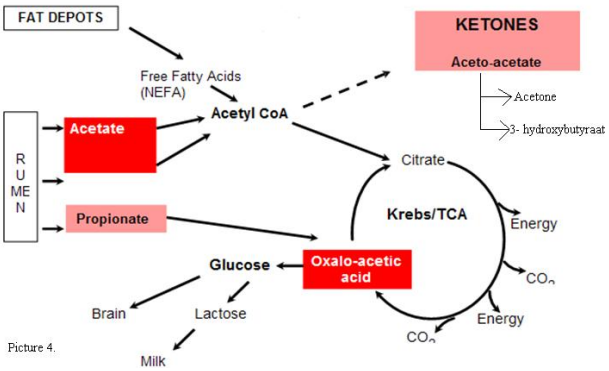
Potassium In this experiment, potassium is one of the most important electrolytes. This element affects nerve and muscle excitability as well as water- and acid-base balance, insulin production, protein metabolism and control of cell pumping processes. Potassium is the major intracellular electrolyte, 98% (100- 160 mmol/L) is situated intracellular and the other 2 % is situated in the extracellular fluid (Suttle, 2010). Plasma references are 3,9 - 5,6 mmol/L (UVDL, University of Utrecht).
Potassium enters the body by digestion and is principally absorbed in the rumen (Coffer et al., 2006). It enters the extracellular fluid via conductance channels in the basolateral membrane of the intestinal mucosa. Potassium is transported across the membrane by the sodium potassium ATP- ase pump (Rule et al., 1985 and Karn et al., 1977).
Low extracellular potassium concentrations cause a depletion in intracellular potassium concentrations, this may be offset by uptake of hydrogen ions, resulting in an intracellular acidosis and an extracellular alkalosis. This effect is reinforced by the kidneys, which need some time to adapt to the situation to spare potassium (Sattler et al., 1998).
Clinical consequences of depriving dairy cows of potassium causes a loss of appetite, loss of milk production and an increase in hematocrit (35,3% to 38,1%) (Suttle et al., 2010, Lima et al., 2005 and Afzaal et al., 2004).
Regulation of extracellular potassium levels begin in the gut, with splanchic sensors which provide an early warning when the potassium amount is lethal (Suttle et al., 2010).
As a response to the sensors the potassium excretion increases by fecal and urinary routes, by the increase of sodium / potassium ATP ase activity and the increasing numbers of pumps in the basolateral membrane of the colon and the distal renal tubule. Tubular reabsorption is restricted in a situation of high potassium levels under the influence of aldosteron. Large amounts of potassium are recycled in the rumen via saliva secretion (Reece et al., 1994).

Sodium and Chloride Sodium and chloride are considered together because of their related metabolism. Serum references for sodium are 135-150 mmol/L, and for chloride 100-110 mmol/L (UVDL, University of Utrecht). Sodium and chloride regulate the acid-base balance, maintain osmotic pressure, and control of extracellular fluid volume (Michell et al., 1995).
Sodium is making up over 90% of the serum base and little is present in the blood cells.
Chloride is found in the gastric goblet cells as HCl and in the extracellular fluid in the form of salts.
Clinical consequences of depriving cows of chloride are; pica, a decrease in milk production and cardiovascular depression (Suttle et al., 2010).
The metabolism of sodium is hormonal and renal regulated. Regulation is achieved in the kidneys by control of reabsorption and secretion in the proximal tubule, by changes in permeability and active transport. The reabsorption in the distal tubule is enhanced with the secretion of aldosteron, so urinary losses decrease when the intake is low. The activation of rennin to form angiotensin II with vasopressin, modulates a response to aldosteron, causing changes in the extracellular fluid volume (Burnier et al., 2007).
Renal chloride reabsorption is a passive process, so the dietary excess of chloride is directly excreted in the urine via the potassium / chloride co-transporter.
Absorption of both sodium and chloride are influenced by each others concentration. The absorption takes place at the intestine, also the active transport is coupled, so the absorption of

one requires the presence of the other. Dietary and endogenous chloride from the rumen is absorbed in the duodenum and exchanged for bicarbonate (Reece et al., 1994).
Much of the sodium that enters the gut originates from saliva, ruminants secrete about 0,31 kg/L bodyweight a day which contains 150 mmol sodium per liter (Suttler et al., 2010).

Calcium Calcium is important for growth and milk production. 99% of the body calcium is found in the skeleton, the other 1 % is an extracellular ion of which 50 % is present in the ionized form and 50 % is bound to serum proteins.
Due to changes in the pH, the balance between ionized calcium and albumin bound calcium shifts. A decrease in pH ensures a shift to the ionized side, an increase in pH a shift to the albumin bound side.
Calcium is used for nerve conduction, cell signaling and muscle contraction (Suttle et al., 2010).
The serum references of calcium are 2,3-3,2 mmol/L (UVDL, University of Utrecht).
Dietary calcium is absorbed by an active process in the small intestine, regulated by hormonally processes, namely by the parathyroid hormone (PTH) and the physiologically active form of vitamin D3 named dihydroxycholecalciferol; calcitriol (Beck et al., 1976 and Lopez et al., 2004).
In a situation of acute calcium deprivation, PTH increases causing bone reabsorption.
An important clinical consequences of calcium deprivation is milk fever, milk fever is mostly seen in high yielding dairy cows, associated with parturition (Reece et al, 1994).

Ketosis Depriving lactating cows of food leads to a severe ketosis (Royce J Treacher et al., 1976). A ketosis takes place when the amount of energy contained in milk and the number of energy required for maintenance is more than the number of energy contained in the food (Reece., 2004). Depriving lactating cows of food results in utilization of body tissues to support milk production.
The decrease in glucose is called a hypoglycemia. This is a condition of an undersupply of propionate, an inadequate rate of gluconeogenesis or a lack of glucose (Baird et al., 1978).
Hypoglycemia leads to a reduction in insulin and a rise in glucagon, this accelerates beta-oxidation, gluconeogenesis and lipolysis in adipose tissue, so body fat is mobilized (Loor et al., 2007).
As shown in picture 4, in the fat depots, lipolysis ensures degradation of triacylglycerol (TAG), which causes the production of free fatty acids, like non esterified fatty acids (NEFA's) (Jorritsma et al., 2003). These amounts increase due to the activated lipolysis. Fatty acids are oxidized in the béta oxidation as an alternative energy source in the cells. The product of this pathway; acetyl coA is transported into the liver to form glucose in the citric acid cycle.



Picture 4.

The liver has a limited capacity to process this acetyl coA and it accumulates in the liver. This accumulation is diverted into other reactions like the formation of ketone bodies in order to make the coenzyme available for further beta oxidation.
Some body tissues can utilize ketone bodies as a source of energy (Royce J Treacher et al., 1976). Unfortunately, there is a limit to the ability of these tissues to utilize ketone bodies, when this limit is exceeded the ketone bodies build up high levels in the blood, milk and urine.
The presence of these high levels in the extracellular fluid reduces blood pH, because ketone bodies are weak acids (Reece et al., 2004).
The liver has also a limited capacity to take up the free fatty acids to form ketone bodies, after

exceeding their oxidation and secretion of TAG in very low density lipoproteins (VLDL) by the liver (Mashek et al., 2004). TAG can accumulate within the hepatocytes resulting in a liver lipidosis (Loor et al., 2007).
The suggestion is that ketone bodies can cause a metabolic acidosis, because they are weak acids. Because of the acidosis, extracellular H^+ ions can be exchanged with intracellular potassium causing a hyperkalemia (Smith et al., 1984).

Material & Methods

Experimental design

The experiment of fasting of dairy cows was used to measure changes in blood concentrations of sodium, potassium, chloride, pO2, PCO_2, glucose concentrations, total protein, hematocrit, beta-hydroxybutyric acid (BHBA), non esterified free fatty acids (NEFA's) and pH.
Blood samples were drawn from day 2 until day 6 at 8.00, 14.00 and 20.00.
Additionally, urine pH, net acid excretion (NAE), specific gravity (S.G), creatinine, potassium and chloride were determined. Urine samples were caught from day 2 until day 5 at 14.00 p.m.
Sampling routine of blood and urine is summarized in table 1.
The duration of the experiment was five days. Day one was used to fit the catheters and for the cows to get used to the situation and acclimatize to the environment. Baseline measurements were performed at the first two days of the experiment before onset of fasting. At day three and day four the cows were fasted. After re-feeding at day five follow up measurements were collected. The cows were allowed to eat at day 5 at 8.00 a.m. At day 6 at 8.00 a.m. the last samples were taken.

	Day 2	Day 3	Day 4	Day 5	Day 6
	Zero measurements	Fast	Fast	Follow up	Follow up
T1: 8.00 Blood sample	Bloodgas values, Total protein, Ht, BHBA	Bloodgas values, Total protein, Ht, BHBA	Bloodgas values, Total protein, Ht, BHBA	Bloodgas values, Total protein, Ht, BHBA	Bloodgas values, Total protein, Ht, BHBA
T2: 14.00 Blood sample	Bloodgas values, Total protein, Ht, BHBA, Glucose, NEFA's	Bloodgas values, Total protein, Ht, BHBA, Glucose, NEFA's	Bloodgas values, Total protein, Ht, BHBA, Glucose, NEFA's	Bloodgas values, Total protein, Ht, BHBA, Glucose, NEFA's	
T2: 14.00 Urine sample	pH, SG, potassium, chloride, creatinine, NAE	pH, SG, potassium, chloride, creatinine, NAE	pH, SG, potassium, chloride, creatinine, NAE	pH, SG, potassium, chloride, creatinine, NAE	
T3: 20.00 Blood	Bloodgas values, Total	Bloodgas values, Total	Bloodgas values, Total	Bloodgas values, Total	

sample	protein, Ht, BHBA	protein, Ht, BHBA	protein, Ht, BHBA	protein, Ht, BHBA	

Animals Five ± 2 years old healthy lactating Hollstein Friesian cows (528,2 ± 44,5668 kg of bodyweight) between 259 – 281 days in lactation were used.

Cow ID	Number in experiment	Cow number	Days in lactation	Body weight in kg
6735 Sonsbeek O	1	66	259	490
6734 Sonsbeek O	2	40	279	490
9263 Honk OS	3	37	281	510
6731 Sonsbeek O	4	53	278	565
6733 Sonsbeek O	5	19	274	586

The cows were housed in adjacent loose housing stalls with sawdust as bedding and had free access to drink water out of automatic water buckets throughout the experiment.

Blood sampling and measurements The cows were fitted with a 3SC Discofix® catheter in the jugular vein, stitched into the skin. During the experiment, all blood was taken from the catheter.
All blood was taken in the same order each time:
1. Flushing catheter with 5 ml sodium chloride with heparin.
2. Drawn 5 ml blood to throw away because of contamination.
3. Drawn 10 ml blood into the heparin tube and / or serum tube.
4. Drawn 2,5 ml blood into the pH syringe.
5. Flushing the catheter with 10 ml sodium chloride with heparin to prevent coagulation in the catheter.
In the pH syringe the blood was drawn for the analysis of blood-gas values and electrolytes. At the same time there was also a 10 ml blood sample drawn into a heparin tube to analyze the total protein concentration, hematocrit and blood glucose. The blood glucose was only measured at 14.00 p.m. with a droplet blood on the Precision Xtra test strip for the Precision Xceed glucose test. After centrifugation of the heparin tubes, the plasma was used to analyze BHBA and creatinine, also a plasma micronic tube was collected as backup.
Daily, at 14.00 p.m. a blood serum tube was drawn for analysis of the NEFA concentrations. During the experiment, 50 ml spontaneous urine was collected at 14.00 p.m. for analysis of the pH, S.G, electrolytes, creatinine and NAE with help of titration. The urine was caught by tickling under the genital organ of the cow.

Analysis Blood gas values, pH, anion gap, partial pressure of CO_2 and O_2 and HCO_3 were directly analyzed in a blood gas analyzer (Rapid Lab 1260). Values of pH, pCO_2 and pO_2 were corrected for rectal temperature.
Glucose was directly measured with the Precision Xceed, references are: 2,5-4,0 mmol/L (UVDL, university of Utrecht).
Hematocrit was measured with a capillary tube, after centrifuging, determined in a Hawskley Micro Haematocrit Reader, references are: 0,21-0,37 L/L (UVDL, university of Utrecht).
Total protein in plasma was determined with a Refractometer, references are: 67-75 g/L (UVDL, university of Utrecht). BHBA, creatinine and NEFA's in blood were analyzed by UVDL, Universities Veterinary Diagnostic Laboratory, Utrecht, references are: BHBA <0, 85 mmol/L, creatinine 88-240 umol/L, NEFA <0,3 mmol/L (UVDL, University of Utrecht).

The pH of the urine was measured with an Inolab WTW series pH meter, references are 7,4-8,4 mmol/L (UVDL, university of Utrecht).
Specific gravity was measured with a refractometer, references are 1.025-1.045 (UVDL, university of Utrecht).
Net Acid Excretion and chloride were determined in Germany
Potassium and creatinine concentrations were analyzed by UVDL.

Statistical analysis

Descriptive statistics were performed in Microsoft Excel.
Electrolytes were corrected for differences in volume. By using an estimated rate of dehydration calculated with the total protein (TP) values at the zero measurement day 2 at 8.00 as a base line.
Dehydration % = (TP Day x Time x – TP Day 2 Time 1:8.00) / TP Day 2 Time 1:8.00 * 100.
The volume correction was applied on chloride, sodium and potassium.

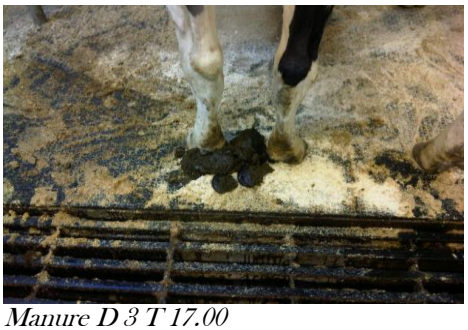
Corrected chloride: Measured chloride time x – [(measured chloride time x / 100%) * percentage dehydration)
Corrected sodium: Measured sodium time x – [(measured sodium time x / 100%) * percentage dehydration)
Corrected potassium: Measured potassium time x – [(measured potassium time x / 100%) * percentage dehydration)

Statistics were assessed by the Statistical Analysis System (SAS). Statistical differences over time were identified using a Bonferroni corrected ANOVA. The ANOVA test for repeated measures was used, P values <0,05 were considered significant. The Bonferroni correction was applied to adjust to the significance level, because more comparisons were determined at the same time. This was applied on differences over time and diurnal effects. P values were dependent on the amount of comparisons, in case of 12 comparisons P values <0, 0042 were considered as significant, and in case of 4 comparisons P values <0,001 were considered as significant.
The Forward Multiple Stepwise Regression Analysis was used to study relationships between variables. P values <0,001 were considered as significant.
Significances in the graphs were shown with the following figures; Significance differences compared with day two were shown with ■, significant differences compared with day three were shown with ●, day four was shown with ♦, and day five with Δ.
Results were presented as mean ± standard error of means.

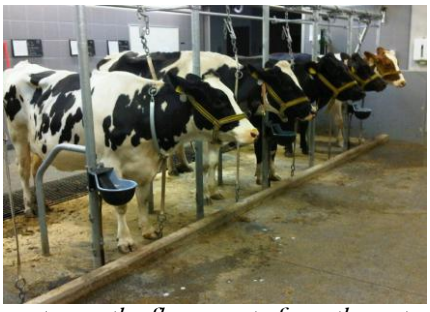
Results

Animals

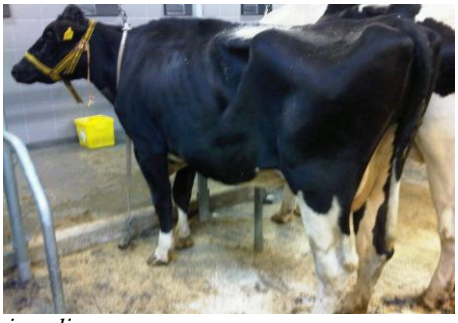
During the acclimatization period on day one, the cows seemed to adapt well to the adjacent loose housing stalls, even so during day two, when the zero measurements were taken. When fasting was initiated at day three cows became more agitated, restless and noisy. Cows started to spill water from the automatic water buckets when food was deprived. When the fasting period ended at day five at 8.00 a.m. and the follow up was initiated, cows were calmer and less noisy. There was also less water near the automatic water buckets.
Cow number one had a diarrhea during the whole week, cow number five had a diarrhea on day six in the morning. The manure was thickened during the fasting period. The rumen groove was invading during the fast and after re-feeding, it filled quickly again.
During the week the extremities became colder. A decrease in milk production was seen during the week.



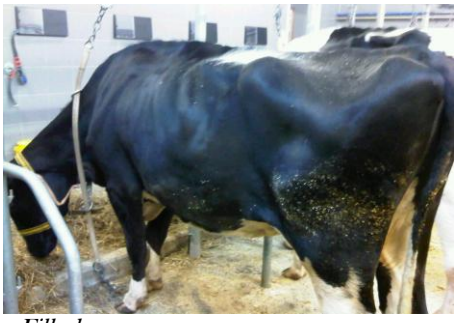
Manure D 3 T 17.00



water on the floor, waste from the water buckets



invading rumen groove
D4 T 8.00 (24 hour fast)



Filled rumen groove
D 5 T 17.00 (Fed)

Blood parameters

Total protein Influences of 48 hour of fasting on total protein concentrations are shown in figure 1. Total protein steadily increases during the period of food deprivation in all cows. Significant increases compared to D2 are detected at D4 (24 hours fasting) and D5 (48 hours fasting), significant increases compared to D3 are also detected at D4 and D5. After re-feeding (D5) total protein decreases within 24 hours.

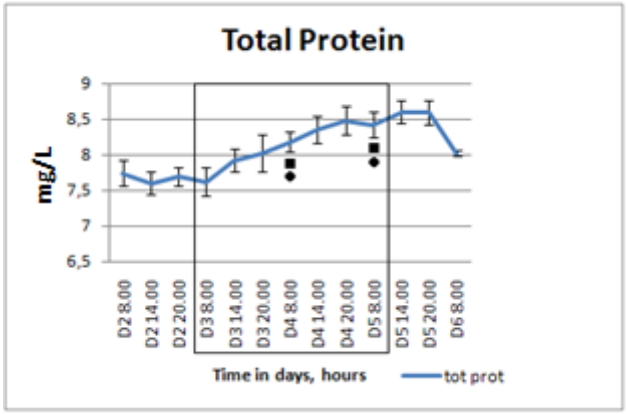


Figure 1. Mean ± SEM plasma total protein concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square. Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between the days are shown with: ■ = significant different from day 2, ● = significant different from day 3.

pH Blood pH concentrations are shown in figure 2. An overall decrease is seen with some peaks. Significant decreases compared to D2 are detected at D4 and D5, compared to D3 at D5 and compared to D4 at D5.

The Forward Multiple Stepwise Regression Analysis shows that the total protein concentration is the most important cause of the change in the dependant variable pH (Atot R2: 0, 3387, P<0, 0001), the other causes are the unmeasured strong ions (R2: 0, 4190 P<0, 0001), the strong ion difference (R2: 0, 0707, P<0,001) and the PCO_2 (R2:0, 0435, P<0, 0001).

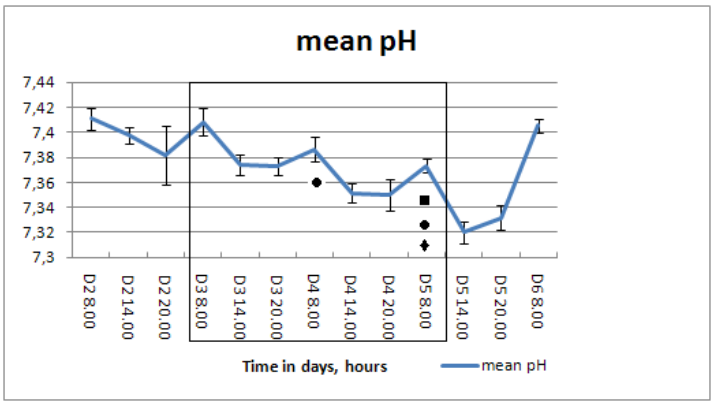


Figure 2. Mean ± SEM blood pH concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square. Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between the days are shown with: ■ = significant different from day 2, ● = significant different from day 3 and ♦ is significant different from 4.

Standard Bicarbonate Figure 3 shows the influence of fasting on bicarbonate; the overall concentration is decreased.

A little tendency in the decreasing concentration is detected with the Bonferroni corrected ANOVA between D2 and D5 (48 hour fasting) and also between D3 and D5. After re-feeding the bicarbonate concentration increases.

The change in HCO (standard) is mainly due to the total protein concentration (R2:0, 3669, P<0, 0001), the unmeasured strong ions (R2:0, 1916, P<0, 0001) and the strong ion difference (R2:0, 1957, P<0, 0001) according the Forward Multiple Stepwise Regression Analysis.

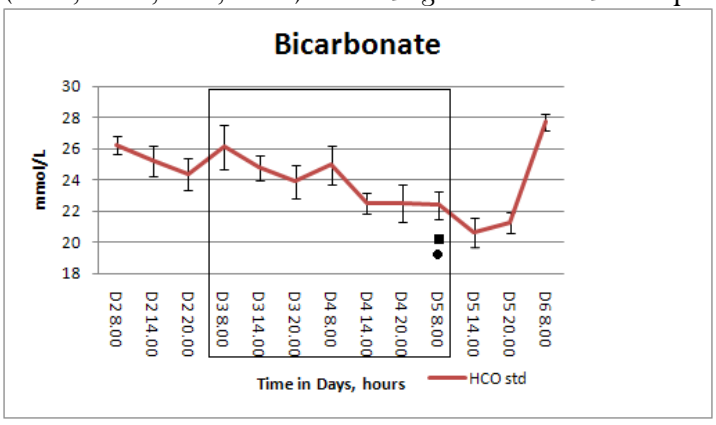


Figure 3. Mean ± SEM blood standard bicarbonate concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square. No significant decrease is seen in the ANOVA test. In the Bonferroni correctied ANOVA a tendency of 0, 0021 is seen between day 2 (■) and day 5. Also between day 3(●) and day 5 is a tendency seen with the Bonferroni p value of 0, 0038.

Anion Gap Changes in the anion gap concentrations are shown in figure 4. There is a significant decrease seen between D2 and D3. Significant increases compared to D2 are detected at D5, there are also significant increases compared to D3 detected at D4 (24 hours fasting) and D5 (48 hours fasting) and compared to D4 a significant increase is detected at D5.

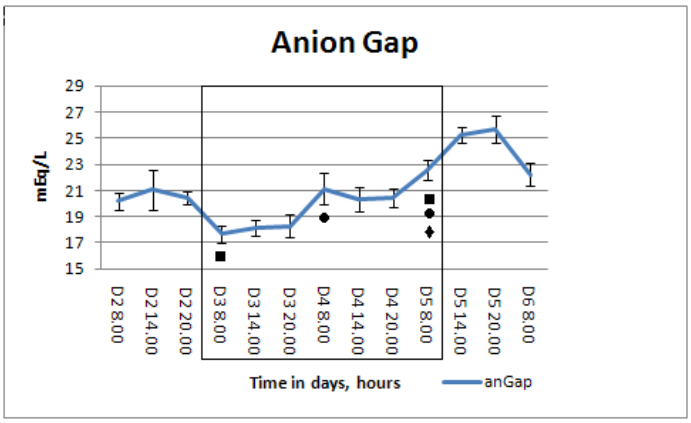


Figure 4. Mean ± SEM blood anion gap concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square. Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between the days are shown with: ■ = significant different from day 2, ● = significant different from day 3 and ♦ is significant different from 4.

Electrolytes

Sodium Influences on the sodium concentrations are shown in figure 5. Significant decreases in actual sodium compared to D2 are detected at D3, and there is a significant change detected between D3 and D5.

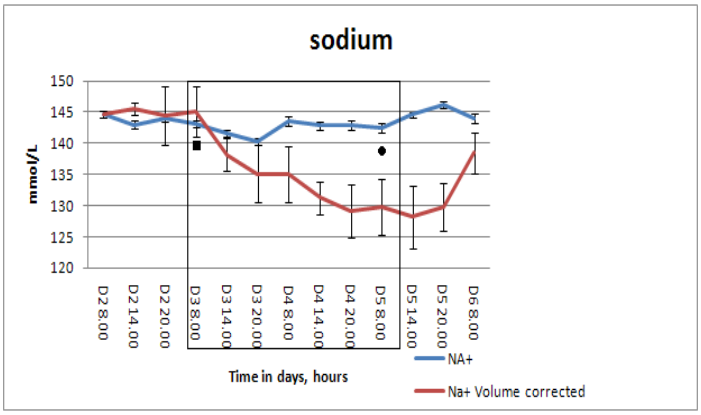


Figure 5. Mean ± SEM blood sodium concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.
Actual changes in electrolyte concentrations are shows with the blue line, volume corrected electrolyte concentrations are shown with the red line. Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between the days are shown with: ■ = significant different from day 2, ● = significant different from day 3.

Chloride Changes in chloride concentrations are shown in figure 6. Significant decreases in actual chloride (blue line) compared to D3 are detected at D5, and between D5 and D6.

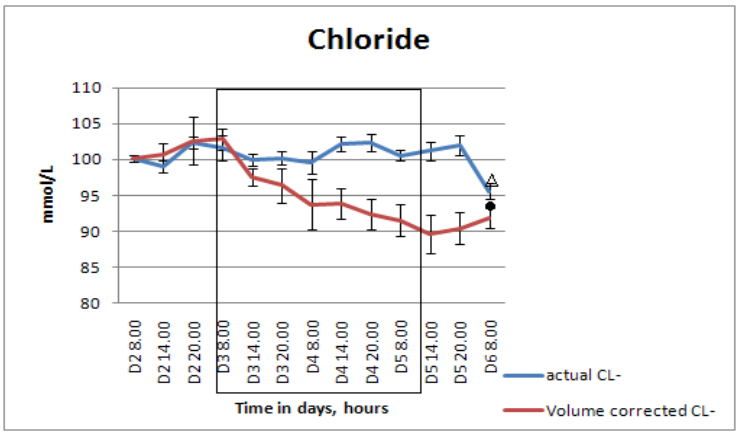


Figure 6. Mean ± SEM blood chloride concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3,

8.00 until day 5, 8.00 is marked with the lined square.
Actual changes in electrolyte concentrations are shows with the blue line, volume corrected electrolyte concentrations are shown with the red line. With the ANOVA test, only a diurnal significant difference is seen. Significant (12 comparisons: Bonferroni corrected p value P<0, 0042) diurnal differences from mean values are shown with: ● = significant different from day 3 8.00 and day 6 8.00. Significant differences between day 5 (Δ) 8.00 and day 6 8.00 are shown.

Potassium The ANOVA test shows no significant changes. Only a diurnal effect is seen with the Bonferroni corrected ANOVA, significant increases compared to D2 at 8.00 are detected at D5 at 20.00, see figure 7.

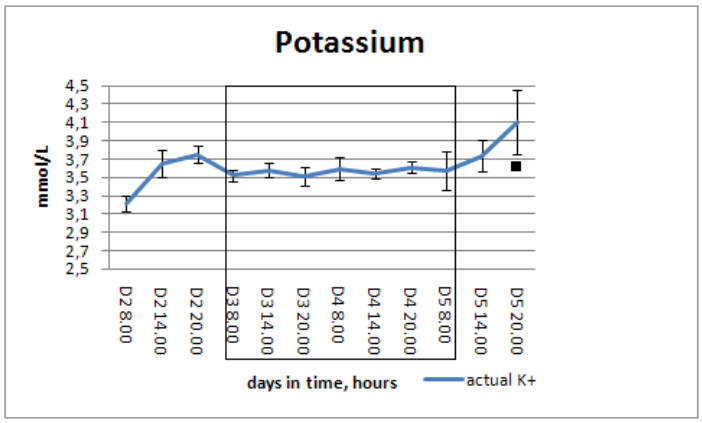


Figure 7. Mean ± SEM blood potassium concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.
With the ANOVA test, only a diurnal significant difference is seen. Significant (12 comparisons: Bonferroni corrected p value P<0, 0042) diurnal differences from mean values are shown with: ■ = significant different from day 2 8.00 and day 5 20.00.

Calcium Figure 8 shows the calcium concentration during the fasting period. There is no significant decrease seen. But according to the Bonferroni corrected ANOVA, there is a tendency seen in the decrease between D3 and D5.

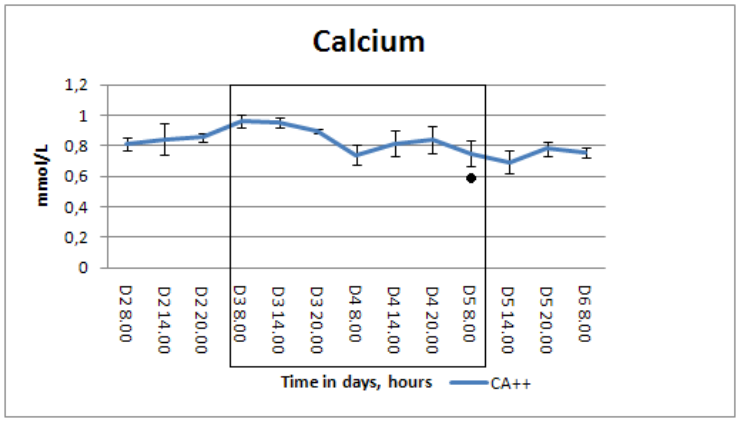


Figure 8. Mean ± SEM blood calcium concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.
There was no significant difference seen in the ANOVA test. But with the Bonferroni correction a tendency of P = 0, 0042 between day 3 (●) and day 5 is seen.

According to the Forward Multiple Stepwise Regression Analysis, chloride does not strictly follow sodium, 20% of the changes in chloride concentrations are caused by the changes of potassium and calcium, due to the acidosis (P<0,001).

Lactate Changes in lactate concentrations are seen in figure 9. Significant increases compared to D3 8.00 are detected at D6 8.00.

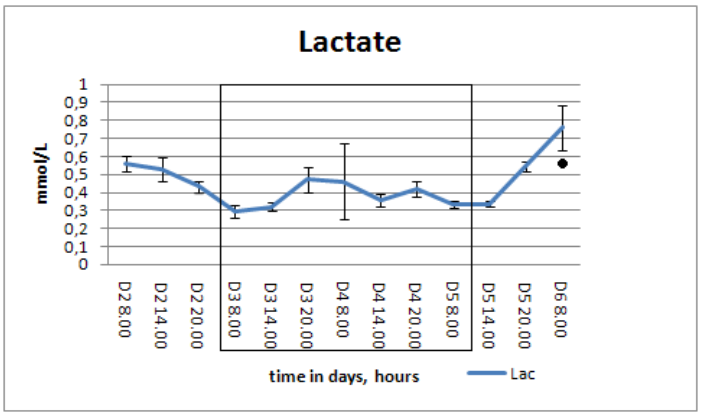


Figure 9. Mean ± SEM serum lactate concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.

A significant (12 comparisons: Bonferroni corrected p value P<0, 0042) different from mean values in time between day 3 (●) 8.00 and day 6 8.00 is seen.

Urine Parameters

Urine pH Influences of 48 hours of fasting on urine pH values are shown in figure 10. Significant decreases compared to D2 are detected at D4 and D5. Compared to D3 a significant decrease is detected at D4 and D5, and there is a significance decrease detected between D4 and D5.

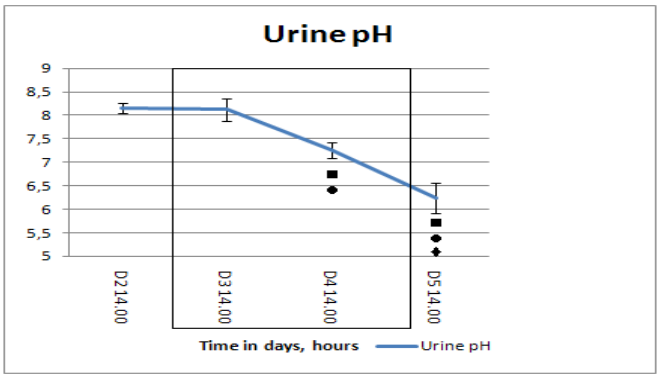


Figure 10. Mean ± SEM urine pH in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.

A significant (4 comparisons: Bonferroni corrected p value P<0,001) different from mean values between days are shown with ■ = significant different from day 2, ● = significant different from day 3 and ♦ differs significant from day 4.

Urine Ammonium Significant increases in urine ammonium concentrations compared to D2, D3 and D4 are detected at D5, see figure 11.

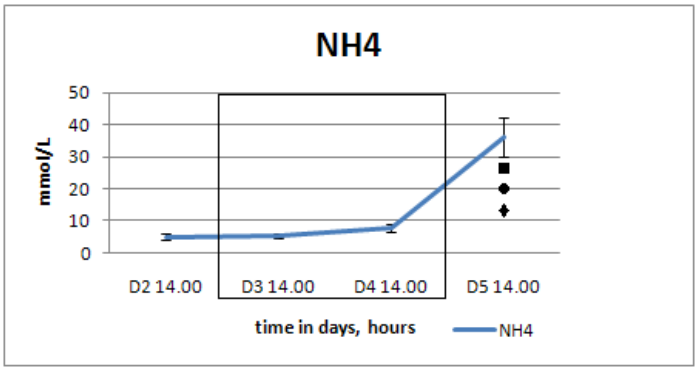


Figure 11. Mean ± SEM urine NH_4 concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.

Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between days are shown with: ■ = significant different from day 2, ● = significant different from day 3 and ♦ differs significant from day 4.

Urine Chloride In figure 12 is seen that significant decreases compared to D2 are detected at D3, D4 and D5. Significant decreases compared to D3 are detected at D4 and D5. There is also a significant decrease between D4 and D5 seen.

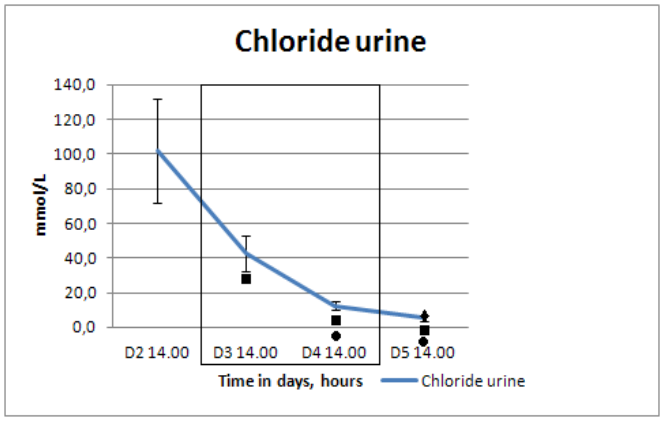


Figure 12. Mean ± SEM urine NH_4 concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.
Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between days are shown with: ■, is significant different from day 2, ●, is significant different from day 3 and ♦ differs significant from day 4.

Urine Potassium/Creatinine Significant decreases in potassium / creatinine concentrations are shown in figure 13. Compared to D2 significant decreases are detected at D3, D4 and D5, significant decreases compared to D3 are detected at D4 and D5.

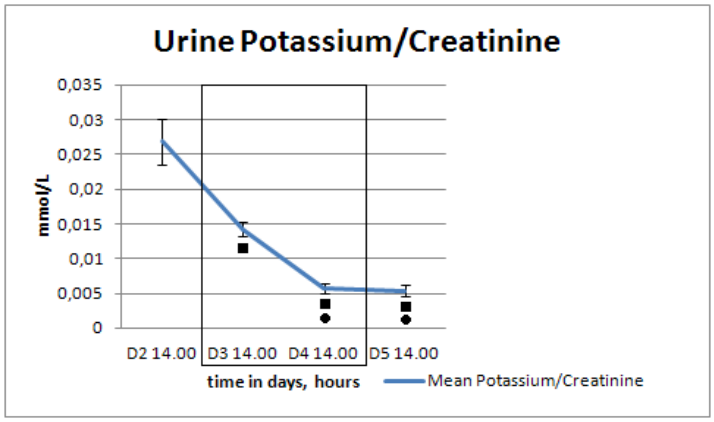


Figure 13. Mean ± SEM urine potassium / creatinine ratio concentrations in n=5 Holstein Friesian dairy cows during the experiment. The fasting period of day 3, 8.00 until day 5, 8.00 is marked with the lined square.
Significant (4 comparisons: Bonferroni corrected p value P<0,001) differences from mean values between days are shown with: ■, is significant different from day 2, ●, is significant different from day 3

Discussion The increase in plasma total protein during the fast, in combination with a mild increase in hematocrit (table 1.) indicates that a mild dehydration occurred. Total protein is not dependent on food intake, so the decreased volume results in an increased total protein. Plasma proteins are nonvolatile weak acids, and an increase in total protein causes an increase in Atot, the main component of Atot are the plasma proteins (>90%) and so resulting in a metabolic acidosis (Muir et al., 1994). During the 48 hour fast, a significant decrease in blood pH, in combination with a proportional decrease in HCO_3 standard and HCO_3 actual, confirms the metabolic acidosis.
Also the Forward Multiple Stepwise Regression Analysis shows that the total protein concentration is the biggest cause of the change in the dependant variable pH (Atot R2: 0, 3387, P<0, 0001). The change in the dependent variable HCO_3 standard is also due to the total protein concentration (R2:0, 3669, P<0, 0001).
The increase in the anion gap concentration is the result of the addition of endogenous acids. The increase in unmeasured anions, is due to the increase in the plasma proteins, which occupy the major anion in the unmeasured anion concentration. Bicarbonate decreases as a result of the consuming buffer reaction with the nonvolatile proteins (Constable et al., 2000).
Electrolyte concentrations in plasma remain constant, probably due to the simultaneous occurrence of the 48 hour fast and the dehydration, this is shown in figure 5 and 6. The volume correction (red line) shows that the sodium and chloride levels should decrease if there was no dehydration. Due to the lack in these electrolytes and the decreased bicarbonate, the renal compensation mechanism is stimulated. Chloride compensates the lack in bicarbonate to neutralize the anion gap, so chloride is maximally absorbed by renal absorption (figure 12). According to the Forward Multiple Stepwise Regression Analysis, chloride does not strictly follow sodium; 20% of the change in chloride is caused by changes of potassium and calcium due to the acidosis (P<0,001). Because the SID and the AG need to be balanced, the buffer base, bicarbonate, is consumed and the acidosis change Ca^{++} and K^{+} concentrations, so chloride has to compensate these changes on the anion side (Constable et al., 2000).
The other electrolytes incorporated in the SID are potassium and calcium. Due to the fast, extracellular potassium decreases and due to the acidosis intracellular potassium is reciprocal exchanged with extracellular H^{+}. Because of the difference in intracellular (98%) and extracellular (2%) potassium concentrations under normal circumstances, there is no obvious extracellular decrease seen, due to the exchange (Suttle., 2010). So in contrast with the hypothesis there is no hypokalemia seen.
Following Sattler et al., 1998 and Peek et al., 2003, the kidneys need 24-48 hour to adjust to the situation, this is in contrast with the outcomes of this study. In figure 14 is shown that there is an immediate reaction to the fast. The kidneys start to reabsorb potassium immediately and so minimal potassium is excreted.
Like the other electrolytes, calcium intake is decreased during the fast.
The acidosis causes a displacement of calcium ions from the albumin binding sites, due to protonation of these sites, and so the extracellular ionized calcium concentration increases (Morse et al., 2007).
Extracellular concentrations of bounded and ionized calcium are balanced. Due to the acidosis this balance shifts to the ionized calcium side, this relatively reduces the decrease, in other words; if there was no acidosis, the measured calcium concentration would have decreased more.
In contrast with the other electrolytes, there is no calcium mechanism which can provide an increase in this short time. The only possibility to save considerable amounts of calcium is reducing milk production. These cows are not in a production state to show an obvious response.

Lactate also shows an increase, but there is no significant change during the fast. In the Forward Multiple Stepwise Regression Analysis, lactate shows no association with the pH or bicarbonate. According to changes in the electrolyte concentrations, there is not yet a mechanism set to solve the dehydration. When such a mechanism would have been stimulated the thirst mechanism and aldosteron production should have been stimulated, resulting in an increase in extracellular volume, this is not seen. Aldosteron also induces potassium excretion in urine, as seen in figure 14, this is not shown (Reece et al., 2004).
Contrary the buffer mechanism reacts immediately to balance the acidosis.
As said before, the decreased extracellular HCO_3^- concentration is a result of the buffer reaction, consuming HCO_3^-.
Due to the acidosis, H^+ has to be buffered in urine. The number of ionized H^+ that can be excreted is minimal. To excrete H^+, it has to be bound to a buffer.
The ammonia (NH_4) buffer is stimulated, shown in figure 11. Glutamine synthesis, which forms NH_4, is stimulated by an acidosis and is active transported into the tubules cells and is deaminated, resulting in 2 NH_4 ions. NH_4 is excreted in the tubular lumen, 'ion trapped', and excreted with H^+ in urine.
With this mechanism a large fraction of the excess H^+ is excreted with NH_4, so the increase in NH_4 (figure 11) shows the stimulation of the ammonia buffer. For every H^+ that is bound by another buffer than HCO_3^- a new HCO_3^- is formed by tubular cells (Barton et al., 2001).

One of the conclusions is that the cows did not drink enough during the experiment. Hypothesis are that the acclimatization period was not long enough to let the cows get used to the little water buckets, at their normal environment they have a bigger bucket. The other hypothesis is that due to the moisture food the cows took before the experiment starts, the alteration takes longer and the thirst mechanism was not yet stimulated.
Through the obvious spilling of water on the ground during the fast, the first hypothesis is more reliable.
The weaknesses of this experiment are the water buckets, in the next experiment other water buckets have to be used or the cows need to acclimatize longer so they get used to small drinking buckets. Another option is to feed them dry hay a couple of days before the experiment so the cows are already accustomed to drink more, and maybe they will do that also over the fasting period.
The aim of the study is not reached, because due to the dehydration which resulted in an acidosis and consequently there was no hypokalemia.

Attachments

Table 1. Blood parameters, mean and standard deviation

Bloodparameters	D2 8.00	D2 14.00	D2 20.00	D3 8.00	D3 14.00	D3 20.00	D4 8.00	D4 14.00	D4 20.00	D5 8.00	D5 14.00	D5 20.00	D6 8.00
PH mean	7,4108	7,3978	7,3818	7,408	7,3744	7,3728	7,3866	7,3516	7,35	7,3734	7,3202	7,3318	7,4056
Standard deviation	0,0199424	0,01525	0,0516	0,02452	0,01843	0,01613	0,02134	0,01662	0,02847	0,01318	0,01994	0,02273	0,01246
pCO2 mmHG mean	42,48	42,50	39,64	42,68	45,90	42,86	43,30	42,52	42,12	39,24	41,70	42,32	46,65
Standard deviation	2,73	2,94	4,05	5,53	2,55	5,11	3,82	1,39	3,71	3,80	4,11	5,53	4,66
pO2 mmHG mean	37	33,7	37,44	37,34	31,9	36,62	37,1	36,34	37,68	37,46	37,16	35,36	33,99
Standard deviation	5,9418011	3,0814	6,42752	1,96418	1,78746	4,39739	4,82856	2,55891	3,58148	2,58902	7,00521	6,57138	3,34167
HCO3 mmol/L mean	27,66	26,62	25,04	27,38	26,98	25,26	26,44	23,86	23,74	23,02	21,9	22,68	29,88
Standard deviation	1,3594116	2,53712	3,08756	4,22043	2,13822	3,47246	3,49113	1,69794	3,48109	2,74718	2,83108	2,54401	2,22868
BE mmol/L mean	2,78	2,28	0,62	2,56	1,40	0,10	1,34	-1,54	-1,66	-1,78	-3,72	-2,88	4,60
Standard deviation	1,6468151	2,3768	2,75536	3,80434	2,04206	2,98245	3,21139	1,77285	3,41072	2,40354	2,60711	2,0969	1,5346
Na mmol/L mean	144,66	142,98	144,1	143,1	141,58	140,26	143,58	142,84	142,94	142,54	144,64	146,22	143,98
Standard deviation	1,2033287	1,38636	1,3	1,37113	1,34796	1,12606	1,91755	1,37768	1,64408	1,73003	0,94499	1,21532	1,66493
K mmol/L mean	3,212	3,656	3,748	3,52	3,58	3,512	3,594	3,548	3,61	3,576	3,736	4,102	3,732
Standard deviation	0,190578	0,33291	0,21242	0,12981	0,18534	0,21347	0,27574	0,12377	0,1437	0,47826	0,37541	0,77889	0,31964
Ca mmol/L mean	0,814	0,844	0,858	0,964	0,952	0,898	0,74	0,814	0,84	0,75	0,694	0,78	0,756
Standard deviation	0,090167	0,22568	0,0638	0,09236	0,06723	0,0295	0,14283	0,19008	0,20543	0,18262	0,16979	0,10296	0,07162
Cl mmol/L mean	100,2	99	102,4	101,6	100	100,2	99,6	102,2	102,4	100,6	101,2	102	95,6
Standard deviation	1,0954451	1,87083	1,81659	3,91152	1,87083	2,16795	3,57771	2,16795	2,60768	1,67332	2,86356	3	2,60768
Lactate mmol/L mean	0,56	0,528	0,432	0,294	0,322	0,472	0,46	0,358	0,418	0,336	0,336	0,542	0,762
Standard deviation	0,102713	0,15287	0,06979	0,07701	0,05541	0,15991	0,47133	0,0726	0,09576	0,04336	0,03847	0,05933	0,28093
Total protein mEq/L mean	7,74	7,6	7,7	7,62	7,92	8,02	8,18	8,36	8.84	8,42	8,6	8,6	8,02
Standard deviation	0,403733	0,35355	0,28284	0,45497	0,35637	0,56745	0,31145	0,4219	0,44385	0,40866	0,36056	0,38079	0,08367
Hematocrit mean	27,4	27,2	27,6	25,6	25,2	27,6	30,6	30	31	29,6	31,2	32,6	33,4
Standard deviation	2,302173	1,64317	2,96648	3,1305	3,03315	3,78153	3,20936	2,54951	4,63681	3,57771	3,63318	4,77494	4,97996
BHBZ mean	1,076	0,662	1,11	0,49	0,354	0,386	0,482	0,614	0,698	0,88	0,714	0,592	0,496
Standard deviation	0,227662	0,1499	0,17234	0,02828	0,06107	0,04827	0,07791	0,18716	0,11862	0,27477	0,11082	0,21948	0,09503
NEFA mean		0,116			0,45			1,192			0,712		
Standard deviation		0,04393			0,1243			0,63857			0,30244		
Strong ion difference mean	47,926	47,952	45,874	45,69	45,79	44,472	47,38	44,644	44,572	45,93	47,534	48,56	52,106
Standard deviation	0,589856	1,53694	2,39469	4,36261	2,37077	2,87152	3,89818	1,43342	3,77645	2,39734	2,13753	2,76824	2,5227
Anion gap mean	20,18	21,04	20,4	17,68	18,8	18,3	21,4	20,32	20,44	22,52	25,24	25,64	22,24
Standard deviation	1,441451	3,33062	1,09545	1,42021	1,37004	1,95959	2,74645	2,10167	1,61802	1,70792	1,24016	2,34585	1,91781
Glucose XCEED mean		4,48			4,22			3,08			3,36		
Standard deviation		0,43818			0,2168			0,47117			0,37815		

Table 2. Urine parameters, mean and standard deviation

Urine	D2 14.00	D3 14.00	D4 14.00	D5 14.00
pH mean	8,158	8,12	7,26	6,248
Standard deviation	0,231776	0,52058	0,37736	0,72178
Specific grafity mean	1,0238	1,0358	1,0136	1,0238
Standard deviation	0,006648	0,02558	0,0077	0,00383
K/creat mmol/L mean	0,0268	0,0142	0,0058	0,0054
Standard deviation	0,007328	0,00239	0,00164	0,00167
Cl mmol/L mean	101,6	42,9	12,3	5,4
Standard deviation	66,83325	23,2944	5,44839	3,16275
NH4 mmol/L mean	5	5	8	36
Standard deviation	2,345208	1,81659	2,94958	13,7295
Base mean	274	252	94	64
Standard deviation	77,330459	103,779	70,2139	27,0185
Acid mean	72	42	23	73
Standard deviation	9,396808	18,636	9,52365	28,8998

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