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Laboratory Analysis of Disorders in Sodium and Potassium Homeostasis in Cattle

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Dedicated to

My parents, husband and my daughters

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List of abbreviations

Abbreviation	Name
%	Percent
°C	Degree centigrade
ADH	Antidiuretic hormone
ATP	Adenosine tri-phosphate
BW	Body weight
CLSI	Clinical and Laboratory Standards Institute
DM	Dry matter content
e.g.	exempli gratia "for example"
ECF	Extracellular fluid
et al.	et alii "and others"
FAAS	Flame atomic-absorption spectroscopy
FAES	Flame atomic emission spectroscopy
FAS	Flame atomic spectroscopy
FIA	Flow injection analysis
FP	Flame photometry
FPHgb	Free plasma hemoglobin
g	Gram
g/l	Gram per liter
GIT	Gastrointestinal tract
ICF	Intracellular fluid
ICP-OES	Inductively coupled plasma optical emission spectrometry
IFCC	International Federation of Clinical Chemistry
ISE	Ion selective electrode
K^+	Potassium
KCl	Potassium chloride
LDA	Left displacement of the abomasum
Max.	Maximum

Min.	Minimum
mmHg	Millimeters of mercury
mmol/kg	Millimole per kilogram
mmol/l	Millimole per liter
Na^+	Sodium
pCO_2	Partial carbon dioxide pressure
PCV%	Packed cell volume
pH	Power of hydrogen
r	Pearson's correlation coefficient
rs	Spearman correlation coefficient
RA	Raw ash
RDA	Right displacement of the abomasum
SD	Standard deviation
SE	Standard error

1 Introduction

Macrominerals form a group of inorganic nutrients required in gram quantities in animal nutrition. The group of macrominerals includes sodium, potassium, magnesium, phosphorous, calcium, chlorine and sulfur. The presence of macrominerals in body tissues and fluids is required for the maintenance of vital physicochemical processes including acid-base balance, osmotic pressure, membrane electric potential and neuromuscular signal transmission (Soetan et al., 2010, Ozcan, 2004). At the Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin, increasing numbers of dairy cows with a hypokalemia syndrome have been diagnosed. The disease syndrome has also been reported in France and the USA. The disease most often affects dairy cows at two weeks and longer after calving and is clinically characterized by anorexia, depression and generalized weakness due to lack of muscle tone with signs of gastrointestinal stasis, ultimately resulting in recumbency and death (Sattler and Fecteau, 2014). Typically the disease affects animals suffering from ketosis. The diagnosis requires examination of blood samples at a laboratory fitted with appropriate instruments to determine the potassium level. In addition, as the pathophysiology of hypokalemia in cows with fatty liver and ketosis has not been fully elucidated yet, research has to focus on the external (extracellular space) and internal (intracellular) potassium homeostasis in post partum dairy cows. Accurate analysis of potassium is a prerequisite for studies on potassium homeostasis in post partum cows, as well as for the tailor-made treatment of diseased animals.

A global consensus exists among laboratories working with human blood regarding the standardized procedure for determination of sodium and potassium levels in blood samples. This standard procedure uses ion selective electrode systems (ISE) and the flame photometric reference method (FP) (CLSI, 2000).

To this end, the first aim of the present study is to apply the standardization process described above for the use in a veterinary laboratory in order to standardize ion selective electrode analysis for the determination of sodium and potassium concentrations in blood samples on basis of the “Golden Standard” flame atomic emission spectroscopy (FAES).

In the case of potassium, the pre-analytical phase (the period starting with the preparation of the patient for the sampling procedure and ending with the arrival of the samples at the laboratory) and the diagnostic process in the laboratory were shown to have great impact on the result reported to the veterinarian.

To this end, the second aim of the present study is focused on the factors that play a role in the preclinical phase (storage duration, ambient temperature and *in vitro* hemolysis), which may affect the results of the laboratory analysis of potassium and sodium in blood samples.

Potassium concentration in plasma, serum and whole blood do not necessarily reflect the total body stores of potassium (Abraham and Lubran, 1981), as most of the potassium in the body is located inside the cell. The approach that uses the erythrocyte as a reflection of the intracellular storage through the measurement of sodium and potassium in plasma samples as well as hemolysates, has not solved the before mentioned problem satisfactorily (Janowitz, 1990, Speich et al., 1987). Skeletal muscle biopsies are considered a reliable reflection of the intracellular pool of potassium (Ladefoged and Hagen, 1988, Sjogren et al., 1987).

Considering the latter suggestion, a third aim of the present study is to obtain first insights into the methodology of muscle biopsy sampling in cattle and in the relationship between the plasma potassium concentration (extracellular potassium pool) and the potassium contents in muscle biopsies (intracellular potassium pool).

2 Literature review

Macrominerals form a group of inorganic nutrients, which are required in fairly large amounts (gram quantities) in the nutrition of animals and man. The group of macrominerals includes sodium, potassium, calcium, chlorine, phosphorus, magnesium and sulfur. Presence of macrominerals in body tissues and fluids is required for maintenance of a broad spectrum of physicochemical processes vital to the living organism, including acid-base balance, osmotic pressure, membrane electric potential and the signal transfer in nerves and muscles (Soetan et al., 2010).

Macromineral disorders, especially those in sodium and potassium homeostasis, can originate from insufficient intake, imbalanced rations, losses and disturbances in the regulatory process that maintain macromineral homeostasis. Macromineral imbalance is regularly observed in cattle as a consequence of disturbances in the acid-base homeostasis, insufficient or excessive consumption, or intestinal and renal losses of these minerals. As a consequence, the dysfunction of various organ systems can occur including the functions of smooth and striated muscles and the central nervous system. A reliable laboratory diagnosis forms the basis for the efficient therapeutic management of such disorders. Determinations of macrominerals in blood samples should precede the intervention in individual cattle and particularly when dealing with a herd problem (Goff, 2006).

Potassium and sodium are macrominerals and the most abundant cations present in the intra- and extracellular fluid compartments of the body, respectively. They are distributed throughout the body fluids and play a key role in all parts of animal life (Sentruk and Cihan, 2004). This study will focus on the laboratory diagnosis of sodium and potassium disorders in cattle.

2.1 Potassium

2.1.1 Characteristics of potassium

Potassium, an alkali-metal represented by the chemical symbol K^+ carries the atomic number Z = 19 in the Periodic Table of the Elements. Pure potassium has first been isolated in 1807. It is

derived from potassium hydroxide and has only one electron in the outermost shell, making the element chemically reactive due to the low ionization energy of that electron. The charge of the nucleus is protected by the core electrons (Tiecke, 2010).

2.1.2 Physiological role of potassium

Potassium is the principal cation in intracellular fluid. It plays a key role in the regulation of osmotic pressure, acid-base balance, the transfer of nerve impulses, muscle contractions, in particular the cardiac muscle, and in a number of cell membrane functions as part of the sodium-potassium pump. Potassium is also required during glycogenesis and supports the transfer of phosphate from adenosine tri-phosphate (ATP) to pyruvic acid. Many basic cellular enzymatic reactions such as the cellular uptake of amino acids, protein synthesis and carbohydrate metabolism also depend mainly on potassium (Goff, 2006, Schaefer and Wolford, 2005).

2.1.3 Potassium homeostasis

According to the National Research Council (NRC, 2000), cattle consume diets that contain enough potassium to meet their body requirements. Potassium is present mainly in the intracellular fluid (ICF) compartment in a concentration of 150-160 mmol/l. It is considered a co-factor of enzymes involved in protein synthesis and carbohydrate metabolism. Potassium is also present in the extracellular fluid (ECF) in a concentration of 3.5-5.5 mmol/l, and plays a fundamental role in the maintenance of osmotic equilibrium and the acid-base balance. The ratio between intracellular and extracellular potassium is essential to maintain the resting membrane potential (Goff, 2006).

Ruminants can tolerate the increase in dietary potassium intake through their ability to use the rumen as an additional reservoir for buffering potassium. After a rapid change from low potassium to a rich potassium diet, the rumen serves as a storage space for potassium until renal excretion has started. Increase in the ruminal potassium concentration results in depolarization of the ruminal apical membrane, with a reduction of magnesium uptake and subsequent hypomagnesaemia, resulting in a disorder with known economic significance (Stumpff and Martens, 2007, Suttle and Field, 1967).

Potassium homeostasis is maintained by the balance between the absorption of potassium from the gastrointestinal tract (GIT) and its excretion by the kidneys and salivary glands. Absorption of potassium takes place primarily in the duodenum through simple diffusion (passive transport), with a considerable absorption in the jejunum, ileum and active transport in the colon (Grunberg et al., 2011, Goff, 2004). The rumen then acts as an additional reservoir for the potassium during increased intake (Stumpff and Martens, 2007), especially when feed (alfalfa and barley silages) contains high potassium concentrations. In turn, during periods of low potassium flow to the intestine or if potassium delivered in high amounts, a small portion of potassium is absorbed in the forestomach (Leonhard-Marek et al., 2010).

Potassium is mainly excreted via the kidneys (approximately 90% of daily loss) and salivary glands under the influence of aldosterone, which regulates the potassium excretion of the kidneys in exchange for sodium ions. The GIT plays a role in potassium excretion (approximately 10%) via saliva and the pancreatic secretions to support the kidneys in preventing hyperkalemia (Grunberg et al., 2011, Goff, 2006, Goff, 2004).

More than 95% of the potassium is reabsorbed through the proximal convoluted tubules of the kidneys, so that urinary potassium is predominantly derived from secretion by cells lining the distal tubules. The rate of potassium secretion is regulated by aldosterone activity as well as by the amount of sodium in the luminal fluid and the rate of urine flow (Reineck et al., 1975). Other mechanisms involved in potassium homeostasis include the shifting of potassium into the intracellular fluid compartment under the effect of insulin and catecholamines, which are normal physiological regulators for short term potassium homeostasis. Insulin increases potassium cellular uptake by increasing the Na^+/K^+-ATPase pump activity, particularly in the liver and skeletal muscles (Goff, 2006).

The acid-base status of the blood affects the urinary potassium excretion. Onset of acidosis leads to a potassium shift from the intracellular to the extracellular space and at the same time a shift of H^+ from the extracellular to the intracellular fluid compartment, with subsequent hyperkalemia. Conversely, alkalosis results in shifting of potassium from the extracellular to the intracellular space, resulting in hypokalemia, while H^+ shifts to the extracellular fluid compartment in order to

restore normal blood pH while simultaneously reducing the renal potassium excretion (Constable et al., 2013, Lunn and McGuirk, 1990, McGuirk and Butler, 1980, Harboe, 1959).

In lactating dairy cows, approximately 13% of the absorbed dietary potassium is secreted in milk. Thus, the rate of occurrence and severity of potassium deficiency is directly related to the amount of milk produced (Ward, 1966). Disorders in potassium homeostasis have clinical effect on the cardiovascular and neuromuscular systems. Potassium channels are the major ion channels that are open in the resting cell membrane. With a small decline in extracellular potassium concentration will dramatically increase nerve and muscle cell resting membrane potential. To this end, triggering of an action potential is hampered which explains the symptoms associated with hypokalemia such as muscle fatigue or flaccidity and neuromuscular weakness. With respect to cardiac functions hypokalemia is conducive to the genesis of reentrant arrhythmias. Hypokalemia also increases threshold potential as well as automaticity, thus providing the context for automatic arrhythmias as well. Lastly, hypokalemia decreases conductivity, which also predisposes to arrhythmias of the reentrant type, which have also been observed in association with the hypokalemic syndrome in cattle (Sattler and Fecteau, 2014, Goff, 2004, Phillips and Polzin, 1998).

2.1.4 Disorders of potassium balance

Alterations in the plasma potassium concentration may result from changes in either the external potassium balance (intake vs. excretion) or in the internal balance (intracellular to extracellular movement). Disorders in potassium homeostasis result in either hypokalemia or hyperkalemia. Changes in the plasma potassium levels do not necessarily reflect the potassium status of the body since the bulk of potassium is stored intracellularly, and the K^+/H^+ shift depends on the acid-base homeostasis. To this end, in neonatal calf diarrhea, hyperkalemia is a result of metabolic acidosis while the body is depleted of potassium (Constable et al., 1998). Disturbances of potassium homeostasis have been shown to alter the resting membrane potential, resulting in clinically significant changes in cellular and organ functions (Schaefer and Wolford, 2005).

2.1.4.1 Hypokalemia

2.1.4.1.1 Definition

Hypokalemia is an electrolyte imbalance frequently observed in cattle. It is commonly encountered as a disorder secondary to anorexia and characterized by a decrease in the serum potassium concentration, reaching values below the lower limit of the reference range (3.5-5.5 mmol/l) (Goff, 2006). Mild to moderate hypokalemia (2.3-3.0 mmol/l) may be associated with symptoms such as muscle fasciculation, whereas obvious clinical signs of neuromuscular weakness and muscle flaccidity are not observed unless serum potassium levels decrease to 2.3 mmol/l and below (Sattler et al., 1998, Sielman et al., 1997). Hypokalemia may result from whole body potassium depletion caused by a decreased uptake and increased losses, respectively, and the redistribution of extracellular and intracellular potassium (Peek et al., 2000).

2.1.4.1.2 Etiology and pathophysiology

The most common causes of hypokalemia are decreased dietary potassium intake, increased renal excretion, shift of potassium to the intracellular space due to disturbances in acid-base homeostasis and hormonal imbalances like hyperinsulinemia, decreased potassium availability in GIT due to intestinal obstruction and increased losses from the GIT in enteritis. Repeated administration of mineralocorticoids might also cause hypokalemia (Hoskote et al., 2008, Coffer et al., 2006, Rastegar and Soleimani, 2001, Sielman et al., 1997, Brobst, 1986). Potassium may also be lost by sweating in the hot season (West, 1999, Beede and Collier, 1986, Mallonee et al., 1985). The pathophysiology of hypokalemia is described in detail below.

A. Decreased dietary potassium intake

Anorexia lasting several days due to illness or gastrointestinal tract problems increases the risk of hypokalemia in cattle through decreased potassium uptake. When cattle suffer from prolonged anorexia, the skeletal muscle tissue is liable to be damaged. As the intracellular potassium pool is reduced severe hypokalemia is causing muscle weakness and even rhabdomyolysis has been reported (Sattler and Fecteau, 2014, Peek et al., 2000, Sielman et al., 1997). High-yielding dairy

cows around parturition predominantly during the first two months of lactation are at greatest risk of developing a hypokalemic syndrome, as they experience negative energy balance because nutrient requirements cannot be met by feed uptake (Sattler and Fecteau, 2014, Peek et al., 2000, Collins and Reid, 1980). Grains contain a relatively low potassium content (compared to forage which contains more than 1-4% of potassium and is considered the main source of dietary potassium intake). To this end, feeding high concentrate diets – especially in the hot season when extra potassium losses occur via the sweat – could contribute to potassium deficiency, accompanied by a decrease in milk yield (Clabough and Swanson, 1989, Pradhan and Hemken, 1968). Heat stress and high production are factors that have been documented to increase dietary potassium requirements due to reduced feed intake and the loss of potassium through milk and sweat (West, 1999, Beede and Collier, 1986, Mallonee et al., 1985).

Prolonged anorexia associated with other disease conditions that either increase potassium losses (i.e. diarrhea and increased renal excretion) or promote the intracellular movement of potassium (i.e. metabolic alkalosis, ketosis and insulin administration) may result in low serum potassium levels associated with muscle weakness and recumbency (Goff, 2006, Peek et al., 2000).

B. Increases in renal excretion of potassium

The administration of large quantities of fluids by infusion therapy, particularly non-potassium containing fluids, will promote renal potassium excretion and therefore predispose a cow to hypokalemia as the rate of renal potassium secretion is balanced by the rate of the tubular fluid flow (Peek et al., 2000).

In healthy animals, secretion of aldosterone, as a normal endocrine response to increased plasma potassium, decreased plasma sodium or low plasma volume, results in elevated renal potassium excretion. Hyperaldosteronism is relatively rare in cattle, however, drugs with glucocorticoid activity which are administered as a therapeutic to stimulate gluconeogenesis in cows exhibiting ketosis in early lactation. If those drugs have also mineralocorticoid activity they will stimulate the potassium urinary secretion. Ketotic cows repeatedly injected with isoflupredone acetate developed hypokalemia due to its mineralocorticoid activity which enhanced the renal potassium secretion. In addition, severe hypokalemia was observed in cows being treated with an intra-

mammary infusion of isoflupredone acetate, due to clinical mastitis (Sattler and Fecteau, 2014, Coffer et al., 2006).

Utilization of non-potassium-sparing diuretics such as furosemide, the most commonly used diuretic in cattle, results in a moderate increase in urinary potassium losses (Goff, 2006, Peek et al., 2000).

C. Potassium shift

The balance between intra- and extracellular potassium concentration may be affected by intravenous treatments with glucose solutions or by the administration of glucogenic precursors such as propylene glycol and insulin. Insulin causes a very rapid uptake of potassium into muscle and liver cells. Endogenous insulin release occurs following intravenous administration of therapeutic agents causing temporary hyperglycemia; dextrose and propylene glycol promote the movement of potassium toward the intracellular space. The administration of exogenous insulin favors hypokalemia in cows with ketosis. Insulin increases the cellular uptake of potassium by increasing the activity of the membrane bound Na^+/K^+-ATPase, resulting in increased potassium movement into the cells, particularly into the liver and skeletal muscles (Goff, 2006, Rastegar and Soleimani, 2001, Peek et al., 2000, Sielman et al., 1997).

Intracellular potassium shift is observed in various clinical conditions in cattle resulting in metabolic alkalosis, among these all disorders that hinder the passage of ingesta through the pylorus or the proximal part of the duodenum. This phenomenon is most commonly observed in the right displacement of the abomasum which is associated with the abomasal reflux syndrome described below (Kuiper and Breukink, 1988, McGuirk and Butler, 1980). Conversely, extracellular shifting of potassium is usually associated with metabolic acidosis (Constable et al., 2013, Goff, 2004, Peek et al., 2000, Lunn and McGuirk, 1990, Watts and Campbell, 1971).

The administration of glucose lowers plasma potassium mainly through an insulin-dependent intracellular translocation of potassium, whereas NaHCO3 causes hypokalemia through hemodilution followed by intracellular translocation of potassium (Grunberg et al., 2011).

D. Gastrointestinal diseases causing hypokalemia

Several bovine GIT diseases including left abomasal displacement (LDA), right abomasal displacement (RDA), abomasal volvulus, or any other condition restricting abomasal pyloric outflow, are associated with mild to moderate hypokalemia (Garry et al., 1988). Failure of abomasal emptying due to abomasal displacement and/or obstruction of the proximal part of the small intestine results in sequestration of large quantities of chloride and H^+ in the lumen of the abomasum. Under normal conditions, these ions pass to the small intestine, where the chloride ions are absorbed and the hydrogen ions from the stomach are buffered by bicarbonate secretion from the pancreas. For this reason, failure of abomasal emptying results in the reflux of abomasal contents into the rumen, where electrolytes and water are sequestered and thus not available to the body. As a consequence of these losses, hypochloremic, hypokalemic metabolic alkalosis develops. This process is called abomasal reflux syndrome which is an equivalent to vomiting in monogastric species. As electrolytes and water are sequestered in the rumen the abomasal reflux syndrome leads to liquefaction of ruminal contents and hypovolemic shock. The fluid containing all electrolytes becomes available for the body as soon as the obstruction is resolved. Abomasal reflux syndrome is mainly caused by a posterior functional or mechanical stenosis at the pylorus region, or a strangulating ileus of the proximal duodenum. Blood gas and electrolyte analysis reveal a hypochloremic, hypokalemic metabolic alkalosis. After correction of the obstacle, the process is reversed to a short-lasting metabolic acidosis due to the enormous amounts of electrolytes and water that are released from the rumen and subsequently pass to the small intestines (Constable et al., 2013, Radostits and Done, 2007, Goff, 2006, Sielman et al., 1997).

Increased potassium loss is an effect of acute diarrhea in all animal species. To this end, whole body depletion of potassium is observed with acute enteritis such as salmonellosis, bovine viral diarrhea and winter dysentery (Peek et al., 2000).

2.1.4.1.3 Clinical symptoms

Hypokalemia syndrome in cattle is characterized by anorexia, decreased passage of feces or even absence of feces, inability to move, recumbency and, in some cases, even colic have been observed (Sattler and Fecteau, 2014, Peek et al., 2000). Mild hypokalemia refers to serum

potassium concentrations within the range of 2.5 - 3.0 mmol/l and signs of muscle weakness, decreased GIT motility, depression and renal dysfunction. The disorder responds well to oral potassium supplementation (Phillips and Polzin, 1998).

In animals with severe hypokalemia, serum potassium levels decrease to less than 2.5 mmol/l. These animals are unable to stand with abnormal neck posture caused by the inability to lift their head from the ground. The motility of the GIT has ceased and the uterine tone is decreased, thus increasing the susceptibility of the affected cows to LDA, metritis and retained placenta (Sattler and Fecteau, 2014, Constable et al., 2013, Peek et al., 2000, Sattler et al., 1998, Brobst, 1986).

Severe hypokalemia has been documented as a hidden cause of muscle weakness in cattle after repeated administrations of a mineralocorticosteroid. Affected cattle are often found in lateral recumbency as they are unable to support themselves in sternal recumbency and are unable to lift their head (Sattler et al., 1998, Sielman et al., 1997).

2.1.4.2 Hyperkalemia

2.1.4.2.1 Definition

Hyperkalemia is an abnormal increase in blood potassium level to above 5.5 mmol/l. Hyperkalemia is a clinically significant problem associated with acid-base and electrolyte imbalances which is frequently observed in neonatal calf diarrhea. The incidence of hyperkalemia in acidotic calves was 8.6 times higher than in non-acidotic calves. The loss of bicarbonate in feces and anaerobic glycolysis, as a consequence of hypovolaemic shock, contribute to acidosis, which subsequently results in hyperkalemia due to the shift of potassium in exchange for hydrogen-ions to the extracellular fluid compartment (Trefz et al., 2013b, Trefz et al., 2013a, Constable, 2003, Constable et al., 1998, Weldon et al., 1992).

Pseudohyperkalemia in serum may occur in animals with thrombocytosis, as a result of the excessive release of intracellular potassium from platelets during clotting or whenever extensive hemolysis is present due to the high potassium content of red blood cells (Goff, 2004, Peek et al., 2000).

2.1.4.2.2 Etiology and pathophysiology

A. Increased potassium intake

This condition is iatrogenic and does not result in hyperkalemia as long as the kidney function is normal. Administration of large doses of potassium chloride (KCl) in dairy cows with hypokalemia resulted in hyperkalemia or even potassium toxicity with symptoms of diarrhea, excessive salivation, muscular tremors of the legs, labored breathing, convulsions and death (Constable et al., 2014, Constable, 2003, Peek et al., 2000, NRC, 2000). A dose of 501 g of potassium chloride given to a cow (475 kg body weight) by use of a stomach tube resulted in cardiac arrest and death. The recommended dose of KCl for treatment of hypokalemia is 0.4 g/kg BW i.e. for a 600 kg dairy cow approximately 240 g of KCl divided into two dosages per day (Constable et al., 2014, Constable, 2003, Sattler et al., 1998).

Due to an antagonistic effect, an increase in dietary potassium intake results in a decrease in magnesium absorption across the rumen epithelium. Oral administration of KCl therefore has the potential to decrease plasma magnesium concentration (Leonhard-Marek et al., 2010, Schonewille et al., 2000).

B. Transcellular potassium shift

Hyperkalemia was attributed to simultaneous acidosis with intracellular buffering of H^+ ions in exchange for potassium and with impairment of the Na^+/K^+-ATPase as the principal underlying pathophysiological mechanism (Constable, 2002, Sweeney, 1999). More recent explanations state that lactic acidosis causes active intracellular flux of lactate and H^+ ions through the monocarboxylate transporter, which results in a decrease in intracellular pH. This effect is counterbalanced by an Na^+/H^+ exchange, making the extra sodium available for the Na^+/K^+-ATPase, which causes a net cellular uptake of potassium (Aronson and Giebisch, 2011).

Hyperkalemia occurs commonly in severe metabolic acidosis, especially in association with insulin deficiency, where potassium is redistributed from the intra- to the extracellular space. As a result, potassium is exchanged with H^+ through the cell membrane to maintain electro-

neutrality (Radostits and Done, 2007, Rastegar and Soleimani, 2001, Adrogue and Madias, 1981).

C. Decreased potassium renal excretion

A decrease in renal potassium excretion is the most common cause of hyperkalemia. In humans, hyperkalemia is observed in many disorders, including mineralocorticoid deficiency or resistance to the effect of aldosterone and the use of an aldosterone antagonist like Trimethoprim preparations (Hoskote et al., 2008, Velazquez et al., 1993). It is a clinical feature observed in anuric or oliguric acute renal failure, chronic renal failure in horses and Addison's disease (hypoaldesteronemia) and accompanies severe diarrhea e.g. caused by *Salmonella Spp*.

Chronic hyperkalemia is also a frequent finding in small animals with renal failure or impaired renal function (Phillips and Polzin, 1998).

2.1.4.2.3 Clinical symptoms

Hyperkalemia is associated with depression, general muscle weakness and disturbance in cardiac conduction, which may lead to lethal cardiac arrhythmia (Goff, 2004, Peek et al., 2000). It is observed at potassium concentrations above 7-8 mmol/l. Hyperkalemia is more life threatening than hypokalemia due to its effects on the cardiac and respiratory muscle functions. The increased extracellular fluid potassium concentration forces potassium into the cells through potassium channels, leading to a slight depolarization that becomes constant and affects the excitability, resulting in fatigue and weakness. In severe hyperkalemia, respiratory muscle weakness can be a life threatening complication, marked bradycardia and arrhythmia can result in sudden cardiac arrest (Hoskote et al., 2008, Radostits and Done, 2007, Goff, 2006). Hyperkalemia requires emergency intervention and treatment aims at the correction of whichever disorder may be hampering potassium excretion (Phillips and Polzin, 1998).

2.1.5 Relationship between muscle potassium and plasma potassium

Due to the fact that potassium is mostly located inside the body cells, the plasma potassium concentration is a poor indicator of the total body potassium status. The plasma potassium level might even give inaccurate and misleading information about the type and magnitude of potassium imbalance in disease. In the opinion of various authors, the intracellular potassium concentration (in erythrocytes or skeletal muscle cells) more accurately reflects the body potassium status and for this reason is more suitable to assess potassium depletion (Sattler and Fecteau, 2014, Johnson et al., 1991). In previous studies, however, the use of erythrocytes for determination of the intracellular potassium pool did not deliver the expected results, due to the wide variation observed in the samples collected from healthy cows (Janowitz, 1990, Speich et al., 1987). Skeletal muscle biopsies, in contrast, are considered a reliable reflection of the intracellular pool of potassium (Constable et al., 2014, Ladefoged and Hagen, 1988, Sjogren et al., 1987). The skeletal muscle potassium content is considered the most sensitive and specific way to assess whole body potassium status (Johnson et al., 1991) and therefore is the "golden standard" for evaluation of the total body potassium content. Moreover, Constable et al. (2014) reported that skeletal muscle is the best tissue to sample because it contains approximately 75% of the whole body stores of potassium.

Skeletal muscle biopsies are considered to provide a reliable reflection of total body stores of minerals with a predominantly intracellular location. Many workers have established the value of muscle biopsy as an aid in the diagnosis and treatment of electrolyte disturbances in humans (Ladefoged and Hagen, 1988). In one study performed in cattle, muscle biopsies were obtained from different body sites, among these samples from the external abdominal oblique muscle, by using a modified (6 mm diameter) Bergström muscle biopsy cannula (Constable et al., 2014). The use of a percutaneous biopsy needle of humans has proved a great value in clinical research and in diagnosis of various diseases. Additionally, this method of sampling is rapid causing only marginally trauma and slight discomfort. For this reason this sort of biopsy is frequently applied in sportsmen and is considered a valuable tool contributing to the knowledge of human electrolyte, carbohydrate, lipid, and protein metabolism (Bergström, 1975).

2.2 Sodium

2.2.1 Characteristics of sodium

Sodium is an alkali-metal that has been isolated in 1807. It is represented by the symbol Na^+ and is placed at position 11 in the Periodic Table of the Elements. Its name originates from soda and its symbol originates from the Latin word Natrium (Tiecke, 2010).

2.2.2 Physiological role of sodium

Sodium is the most abundant cation in extracellular fluid and is responsible for the maintenance of the osmotic pressure of the ECF. Normally it is present in serum in a concentration ranging from135-155 mmol/l, according to Rosenberger (1990), and in a concentration of 140 mmol/l, according to NRC (2000).

The main physiological function of sodium is the maintenance of the osmotic pressure in the fluid compartments of the body through regulation of plasma water volume. As part of the "strong ions", sodium is involved in the regulation of the acid-base balance and contributes to nerve and muscle functions through maintenance of membrane potentials and transmission of nerve impulses. In addition, sodium is involved in Na^+/K^+-ATPase, which is responsible for maintaining the electric gradients required for nutrient transport over membrane borders. Sodium also plays a role in the absorptive processes of monosaccharides, amino acids, pyrimidines and bile salts (Thompson and Hoorn, 2012, Sentruk and Cihan, 2004). Sodium constitutes a major component of saliva and - together with bicarbonate - serves to buffer acids that are generated during ruminal fermentation (Blair-West et al., 1970).

2.2.3 Sodium homeostasis

Sodium is the main cation present in the ECF compartment with approximately 1/2 to 1/3 of body sodium in the form of available and exchangeable sodium. The remaining sodium is then bound in bone substance (McKeown, 1986). The main absorption site of sodium is the GIT. Active and passive transport mechanisms are present in the reticulorumen, omasum, abomasum and the

intestines (Renkema et al., 1962). Normally, sodium is secreted in milk with 25 to 30 mmol/l and increased losses occur in cows suffering from mastitis, due to the leakage of serum constituents into the milk (Schellner, 1971).

The regulation of plasma sodium depends on the water balance. Since the exchangeable sodium content is the principal determinant of ECF volume, the sodium deficit is the principal cause of decreased ECF volume and increased sodium content then results in increased ECF volume (Dow et al., 1987, Saxton and Seldin, 1986, McKeown, 1986).

The kidney plays a key role in the maintenance of ECF volume, as osmoreceptors and baroreceptors located in the kidney steer the sodium homoeostasis. In the case of hyperosmolality (hypernatremia), osmoreceptors stimulate antidiuretic hormone (ADH) secretion from the pituitary gland and stimulate thirst, which then results in increased water intake and water retention by the kidney. To this end, increasing the water content therefore results in the lowering of the sodium concentration and a subsequent return to normal. Opposite changes occur with hypo-osmolality. With the presence of hypovolemia (decreased ECF), baroreceptors stimulate the renin-angiotensin system, resulting in aldosterone release from the adrenal cortex. Hyperaldosteronism lead to reabsorption of sodium, chloride, and water (Bohn, 2012).

2.2.4 Disorders of sodium balance

Changes in serum sodium concentration depend on changes in the water balance (Leaf, 1962). An abnormal decrease in serum sodium level (less than 132 mmol/l) is termed hyponatremia while, increases in sodium levels in serum (above 152mmol/l) characterize hypernatremia. Disturbances of sodium balance affect the osmolarity and can result in fatality e.g. in calves with neonatal diarrhea (Byramji et al., 2008, Radostits and Done, 2007). Commonly, green forage does not contain sufficient quantities of sodium to meet the dietary needs of a dairy cow. This lack is compensated for by the addition of sodium chloride as common salt to the diet or by allowing the animals to consume salt ad libitum (Hays and Swenson, 1985). In lactating dairy cows, the most common causes of chronic sodium depletion is feeding on a low salt diet and losses of sodium in the milk which is even increased in cows under stress or cows suffering from mastitis (Michell, 1985).

2.2.4.1 Hyponatremia

2.2.4.1.1 Definition

Hyponatremia is defined as a reduction of serum sodium concentrations to levels below 132 mmol/l. The condition is often associated with disorders causing sodium depletion and with decreases in the circulatory volume. Such conditions include vomiting in monogastric animals, excessive sweat losses, renal and adrenal insufficiency (Carlson and Bruss, 2008). Hyponatremia is commonly observed in calves with acute diarrhea (Lorenz et al., 1998).

2.2.4.1.2 Etiology and pathophysiology

Hyponatremia is considered a consequence of relative water excess, resulting from retention of free water or, conversely excess sodium loss in the body.

A) Excessive water intake

Hyponatremia is associated with excessive water intake, which results in increased urine excretion and simultaneous decreased renal sodium absorption. Hyponatremia has been observed in large breeds of dogs with psychogenic polydipsia where the rate of water consumption exceeds the renal capacity for free water clearance (Tyler et al., 1987). Also in calves and adult cattle under experimental induction of water intoxication, a sharp drop in plasma Na^+ concentration due to haemodilution has been reported (Njoroge et al., 1999).

B) Renal losses

Renal sodium losses due to increased renal excretion are caused by failure of hormonal control including inappropriate production of ADH or failure to produce aldosterone or cortisol. The inappropriate release of ADH results in high losses of sodium in urine even in presence of hyponatremia. This condition is observed in dogs of large breeds as a consequence of psychogenic polydipsia, in heartworm infection and neoplasia. In addition, the use of antidiuretics as well as the administration of hypotonic solutions at infusion results in release of

ADH with subsequent excessive water retention without the development of significant sodium depletion or decreases in the circulating fluid volume (Radostits and Done, 2007, Adrogue and Madias, 2000, Worth, 1988).

C) Other causes

The most common causes of sodium depletion are associated with inadequate dietary sodium intake, a decrease in sodium absorption and excessive loss of sodium-containing fluid (McKeown, 1986), in addition to losses from GIT through vomiting or diarrhea (Lakritz et al., 1992). Hyponatremia is observed in patients suffering from ascites, peritonitis and ruptured bladder. These conditions are termed "third space problems", as they result from the accumulation of sodium-containing fluids within the body cavities e.g. in case of rupture of the bladder. The process is driven by the concentration gradient between the blood and the fluid present in the body cavity (Rose and Karus, 2013, Byramji et al., 2008).

D) Pseudohyponatremia

A pseudo decrease in sodium concentration is observed when using flame photometry in the presence of marked hyperlipemia or hyperproteinemia. Whenever excessive fat and protein, respectively, occupy part of the serum phase or plasma phase, analysis of centrifuged samples result in falsely low concentrations of the electrolytes which are only dissolved in the aqueous phase. This problem can be avoided if direct ion selective electrode is used for electrolyte determination without previous sample dilution (Fortgens and Pillay, 2011).

2.2.4.1.3 Clinical symptoms

The most obvious clinical findings after having fed diets extremely low in sodium content or even without any supplemental sodium chloride to dairy cows are manifested within 2-3 weeks as pica, drinking urine of other cows, licking and chewing various objectives (Lorenz et al., 1998).

Hypotension, peripheral circulatory failure, muscular weakness, hypothermia and marked dehydration are common findings and result finally in renal failure (Radostits and Done, 2007, Adrogue and Madias, 2000).

2.2.4.2 Hypernatremia

2.2.4.2.1 Definition

Hypernatremia is the elevation of the serum sodium concentration above152 mmol/l and usually is associated with increased serum osmolality and consequently dehydration (Carlson and Bruss, 2008).

2.2.4.2.2 Etiology and pathophysiology

A) Excessive water loss

Hypernatremia occurs in dehydrated animals when water loss exceeds sodium losses. It mainly occurs in the initial stages of diarrhea, vomiting or renal disease (Saxton and Seldin, 1986). Hypernatremia also develops as the result of water loss in panting animals via the evaporative respiratory water loss and in diabetes insipidus due to excessive renal water loss due to lack of ADH with restricted water intake (Breukink et al., 1983, Carlson et al., 1979).

B) Salt gain

Hypernatremia occurs in cattle fed on a high salt diet, especially containing sodium chloride, whenever water allowance is restricted and leads to sodium toxicity. High amounts of sodium chloride in the diet have been shown to increase the incidence and severity of udder edema (Angelos et al., 1999, Pearson and Kallfelz, 1982). In addition, drinking water containing high concentrations of sodium chloride taken up by growing cattle resulted in hypernatremia and toxicosis (Michell, 1985, Randall et al., 1974).

C) Other causes

Sodium excess can result from administration of excessive sodium-containing fluids to patients with severely compromised renal function (Michell, 1985). In addition, hypernatremia transiently occurs after intravenous administration of hypertonic solutions of saline or sodium bicarbonate.

2.2.4.2.3 Clinical symptoms

Severe hypernatremia, (sodium chloride intoxication) in which serum sodium concentration was 176 mmol/l manifested itself clinically by depression, weakness, dehydration and diarrhea (Pringle and Berthiaume, 1988). Signs of severe anorexia, reduced water intake, weight loss and physical collapse were observed in growing cattle maintained on water that contained 12 to 25g/l sodium chloride (Weeth and Haverland, 1961).

Muscle rigidity, twitching and tremors, ataxia, opisthotonus and intermittent convulsions were observed in Holstein beef cattle with a history of salt poisoning resulting in central nervous system dysfunction caused by loss of water from the brain cells (Sentruk and Cihan, 2004, Carmalt et al., 2000).

Owing to the great clinical importance of potassium and sodium in cattle accurate determination of these electrolytes is needed. To this end laboratories have to be fitted with appropriate instruments to determine potassium and sodium contents in various substrates among these blood, saliva and urine. The pre-analytical phase (that is the period starting with the preparation of the patient for the sampling procedure and ending at the arrival of the samples at the laboratory) and the diagnostic process in the laboratory were shown to have great impact on the result that will finally be reported to the veterinarian.

2.3 Pre-analytical factors

The quality of the analysis of blood samples in certified laboratories has improved due to high standards applied for quality control. Pre-analytical factors that exert an effect on the result of laboratory analysis still form a problem in todays' veterinary practice. Pre-analytics refers to all

processes that occur prior to the actual laboratory analysis, starting from sample collection (e.g. blood, saliva or urine), to sample stabilization, transport, and storage. Numerous pre-analytical factors may affect the results of clinical laboratory tests, among these the choice for the tube and the site for the collection of blood, the preparation of the sampling site, the way the samples are obtained as well as the storage and transport conditions. The latter factors have been demonstrated to lead to false interpretations by the veterinarians treating the patient (Asirvatham et al., 2013, Humann-Ziehank and Ganter, 2012, Schulze, 2008). The pre-analytical factors are listed in Table 1.

Table 1. Overview over pre-analytic factors that affect the quality of the blood samples arriving at a laboratory

Factor	Species	Reference
Delayed separation and storage temperature	Human	(CLSI, 2000)
	Human	(Beaulieu et al., 1999)
	equine, bovine, ovine, and canine	(Szenci et al., 1991)
	New born infants	(Paerregaard et al., 1987)
	Rat	(Brito et al., 2008)
	Cattle	(Schulze, 2008)
In vitro hemolysis and hemolysis during sample transportation	Human	(CLSI, 2000)
	Human	(Frank et al., 1978)
	Cattle	(Schulze, 2008)
Collection tubes and handling of samples	Human	(Lippi et al., 2005)
	Human	(Almagor and Lavid-Levy, 2001)
	Cattle	(Schulze, 2008)
	Human	(Junge et al., 1978)
Cellular leakage	Human	(Asirvatham et al., 2013)
Contaminants and anticoagulants	Human	(Asirvatham et al., 2013)
	Human	(Koch and Cook, 1990)
	Cattle	(Schulze, 2008)

2.4 Standard test methods for determination of sodium and potassium in body fluids

Over the last decade, there is increasing need for fast analysis of blood samples or even bedside (cow-side) testing to improve and accelerate the diagnostic procedure in patient care and improve the art of diagnosis with regard to simple, speedy and fully automated analysis techniques (Albert et al., 2011).

Various analytical procedures are available for determination of potassium and sodium contents in various substrates among these flame photometry (FP), indirect ion selective electrode (ISE) and direct ISE use. Flame photometry has been traditionally used for determination of total potassium and sodium concentrations in blood samples. As only specified laboratories are equipped with a flame photometer, samples have to be properly sampled, processed, packaged, and posted to the lab. Results will not be available earlier than at two or three days after sampling. In addition, hemolysis due to improper processing or handling of tubes during transport has been shown to affect the results, giving rise to misinterpretation (Asirvatham et al., 2013). Also, the alterations of lipid and protein concentrations have been shown to affect the outcome of electrolyte measurements obtained by FP in man and other animal species due to alterations of the serum water content where, the measurements of electrolytes occur in the water phase of the specimens. These disadvantages were overcome recently by introducing the direct ISE into the veterinary laboratories which measure the ionized form of the elements regardless to the water phase of the specimens. Consequently, there is no effect of lipids and proteins on the outcomes of direct ISE measurements. Thus, direct ISE measurements of potassium and sodium ion concentrations reflect the activity of these ions and therefore the measurements are more relevant than the results obtained from samples after dilution (Fortgens and Pillay, 2011, Albert et al., 2011).

2.4.1 Instruments for measurements of potassium and sodium

2.4.1.1 Flame atomic spectroscopy

The Flame atomic spectroscopy (FAS) covers three types of optical principles which are absorption, emission, and fluorescence spectroscopy.

2.4.1.1.1 Flame atomic emission spectroscopy

Flame atomic emission spectroscopy (FAES) is considered one of the oldest referenced laboratory methods used for measuring the concentrations of total potassium and sodium and other metallic elements (lithium, cadmium, copper and barium) in plasma, serum and urine samples. It forms a subgroup of emission photometry. The measurements by FAES obtained after introducing the sample into the flame as a sprayed solution and the heat from the flame evaporates the solvents with breaking the chemical bounds to create free atoms. Afterwards, the flame provides thermal energy to the elements leading to the excitement of their valence electrons. When the electrons return back to their basic state, they emit energy of a particular wave spectrum which is characteristic for each element (Stahlavska, 1973).

Various advantages are related to FAES including wide dynamic ranges, high capability of simultaneous multi-element determinations (Kaga et al., 1997, Ramirez-Munoz, 1970). FAES has disadvantages as it requires manual operation, costly instrumentation and time (Albert et al., 2011, Hald, 1947). In addition, the analysis by flame photometer requires a preceding dilution procedure of the materials.

2.4.1.1.2 Flame atomic-absorption spectroscopy

Flame atomic absorption spectroscopy (FAAS) is considered a highly selective method for electrolytes analysis. The basic principle of measurements by FAAS was established since the second half of the 19th century and it was clear and simple. A hollow-cathode lamp contains the element to be analyzed and emits radiation characteristic for these elements. The radiation from the lamp is absorbed by the ground stated element and in consequence a reduction of hollow-cathode lamp radiation is measured. A monochromator isolates the desired wave length and allows it to fall on a photomultiplier followed by generation of electrical signals which result in electronic output proportional to the analyte atoms (Van Loon, 2012). Flame atomic absorption spectroscopy has the advantages of high selectivity and sensitivity, effective background correction and no spectral interferences (Ramirez-Munoz, 1970).

2.4.1.1.3 Flame atomic fluorescence spectrometry

Flame atomic fluorescence spectrometry is a method of flame spectrometry analysis in which the intensity of fluorescent emission is measured after atoms have been excited by absorption of flame energy. The fluorescence technique has several advantages compared to absorption and emission technologies which make the method a valuable tool for chemical analyses. In addition, atomic fluorescence spectrometry proved superior to either atomic emission or atomic absorption flame spectrometry in terms of simplicity, speed, cost and environmental protection (Musil et al., 2014, Wu et al., 2012, Kirkbright, 1971).

2.4.1.2 Ion selective electrode

Ion selective electrode (ISE) is a relatively simple membrane based potentiometric device that converts the activity of a specific ion dissolved in a solution into an electrical potential dependent on the logarithm of the ionic activity, as firstly described by Nernst (Morf et al., 1975). The ISE technology is largely used in biochemical and biophysical research, where measurements of ionic concentrations in a solution are required allowing for the selective measurement of a wide variety of cations and anions (e.g. Na^+, K^+, Ca^+, Ag^+, Cl^-, Br^-, F^- and organic ions) (Covington and Bates, 1973). The sensing part of ISE is usually made as an ion-specific membrane, along with a reference electrode. The measurement with ISE is conducted in a two electrode cell, one is the reference electrode and the other is the indicator electrode. An electrical potential is generated by the difference between the two electrodes. The output potential is proportional to the amount or concentration of the selected ion in an aqueous solution. The measured electrode potential (E), is related to the activity of an ionic species by the Nernst equation.

$$E = Eo + 2.3\frac{RT}{nF}\ Log\ Activity$$

Where:
Eo = a constant for a given cell
R = the gas constant
T = the Temperature in Kelvin
n = the ionic charge
F = the Faraday constant

The expression $\frac{RT}{nF}$ is termed the Slope Factor

Ion selective electrode measures the activity of an ion rather than its concentration. This is in contrast to FP, where the total amount of a substance is retrieved either present as an ion or in a bound form (Figure 1). Thus the results obtained by ISE differ from those obtained by FP (Sharma and Sarmah, 2013, Maas et al., 1985). Direct ISE is used for blood gas analysis and by most other point-of-care analyzers whereas indirect ISE and flame photometry require a dilution step and are used mainly in high-throughput commercial laboratories. In critically ill patients there is often a difference in the results between direct ISE on one hand and indirect ISE and flame photometry on the other. These differences are due to the electrolyte exclusion effect. This effect is caused by the solvent-displacing effect of lipids and proteins in plasma and has been shown to cause a difference of approximately plus 7% between the results obtained by direct ISE according to the consensus of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2000). The results for samples with normal concentrations of plasma water, lipids, total protein, pCO_2 and pH, however, were demonstrated to deliver no difference of clinical relevance when compared to results of flame photometry (Fortgens and Pillay, 2011, Maas et al., 1985, Aw and Kiechle, 1985, Buckley et al., 1984, Levy, 1981, Ladenson, 1977). ISE measures the ion activity which represents the physiologically active part of the substance. In order to make the results obtained by ISE comparable to those obtained by methods that require a dilution step, a correction factor is applied directly by the machine (Maas et al., 1985). The correction factor is based on the primary reference solutions with an ionic strength of 160 mmol/l at 37°C, which provides an activity coefficient similar to that in normal human plasma and serum.

Laboratory analysis on basis of ISE technology recently has found its entrance into veterinary practice. The latter technology allows accurate and precise determination, either at or near the bedside of several analytes including blood gas analysis, Na, K, Cl, ionized Ca, glucose and lactate and is used for point-of-care-analysis in humans most often at intensive care facilities of hospitals (Burritt, 1990). In veterinary practice this technology is frequently used for blood gas analysis. Small hand-held devices, relying on ISE technology, have become available that can be used as "cow-side" test in the stable.

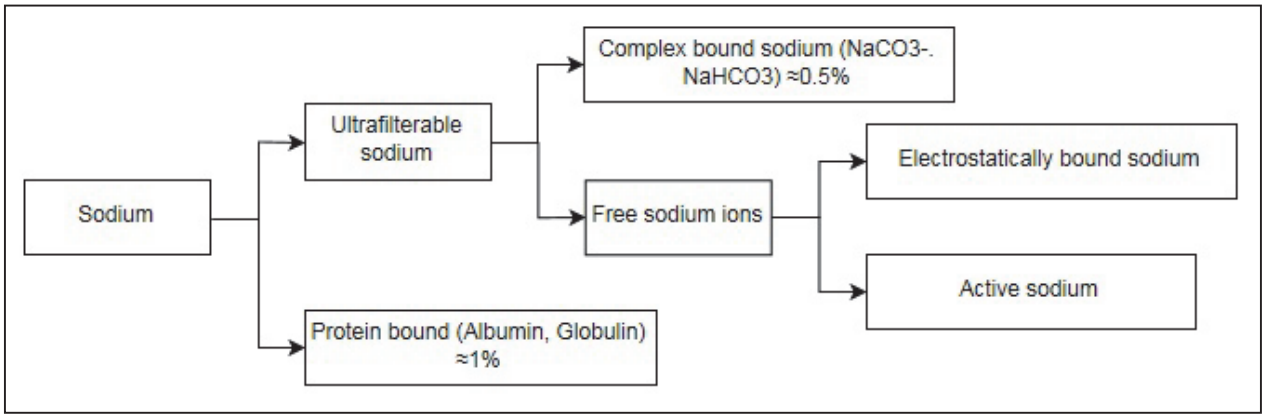

Figure 1. Sodium fractions of blood plasma modified after Maas et al. (1985)

The use of ISE technology becomes more and more popular for the determination of electrolyte concentrations in various materials. ISE can be used as a detector in a number of practical applications and for high scale determination of electrolytes in physiological samples. In addition, ISE technology does not require too much time, and no dilution steps have to precede the measurements as the whole blood can be used directly. Although the ISE is very selective, it is not free of interferences. Method interference may be caused by the effect of any species in the sample solution or process that precipitates, complexes, oxidizes or reduces the ion being measured and could lead to a false result. There are many sources of interference and the staff members of a laboratory have to be familiar with these obstacles in order to avoid inappropriate interpretation and subsequent misdiagnosis by the veterinarian (Table 2).

ISE are classified according to the nature of the basic membrane material into solid, liquid and glass membrane electrodes. The most useful membranes are often solid or liquid electrolytes as they are composed of partially or completely ionized acids, alkalies or salts (Chandra and Lang, 2006).

2.4.1.2.1 Solid-state membrane electrodes

Solid membrane electrodes are membranes composed of solid salts, which may be single crystals or polycrystalline pellets (Chandra and Lang, 2006, Vytras, 1989).

2.4.1.2.2 Liquid ion selective electrode

A liquid ion selective electrode consists of membranes made from liquid electro-active substances dissolved in a mediator (water-immiscible solvent) or in polymeric supports with gelling agents (plasticizers). The latter electrode is the most common type of liquid electrodes used. The electro-active materials used in the membranes are either ion-exchangers (acids, bases and salts), or neutral carriers (valinomycin, nonactin and various crown ethers) (Chandra and Lang, 2006, Vytras, 1989).

2.4.1.2.3 Glass membrane electrodes

Glass membrane electrodes are considered the best and the oldest ion selective electrodes and are made of various multi-component glasses (Vytras, 1989).

2.4.1.3 Inductively coupled plasma spectrometry

Inductively coupled plasma optical emission spectrometry (ICP-OES) has been used for the determination of trace elements in a numerous materials. The principle of measuring by ICP-OES technique is based upon the spontaneous emission of photons from the excited atoms produced by radiofrequencies discharge. After injecting the sample solution into the central channel of the instrument, the solution is converted to an aerosol, which quickly evaporates by adjusting the plasma temperature to a high temperature (10 000 Kelvin) and liberating the elements as free atoms in the gaseous state. The plasma imparts additional energy to the atoms, promoting them to excited states. The atoms then return back to the ground states after the emission of photons. These photons have a characteristics wavelength specific to each element and the total number of photons is directly proportional to the concentration of the originating element in the sample. A portion of these photons emitted by the ICP is collected with a lens and forms an image of the ICP on a monochromator. The particular wavelength exiting the monochromator is converted to an electrical signal by a photodetector. The signal is then amplified and processed by the detector electronics, displayed and stored on a personal computer. The main analytical advantage of the ICP is the possibility of using high temperatures (7000–8000 K) which results in lowering the chemical interferences, high electron density, considerable degree of ionization for many

elements, simultaneous multielement capability (over 70 elements), low background emission, and relatively low chemical interference and high stability leading to excellent accuracy and precision (Ojeda and Rojas, 2007, Stefansson et al., 2007, Bauer et al., 1991).

2.4.1.4 Flow injection analysis

Flow injection analysis (FIA) technique is an automated method in which a sample is injected in a small volume into a flowing stream of a carrier substance that mixes with other continuously flowing solutions before reaching a detector, further monitoring the products of the reaction before a steady state condition is reached. In many cases, the result is obtained within 20-30 seconds of injecting the sample (Rocks and Riley, 1982, Stewart and Rosenfeld, 1981). FIA can be implemented for the determination of numerous liquid-phase compounds, as it employs direct physical measurement. It is unlikely to replace established techniques (e.g. electrochemistry, atomic absorption and emission spectroscopy) because it is not a simple technique (Broughton et al., 1983, Ruzicka and Ramsing, 1982).

Table 2. An overview of the interferences of relevance using ISE technology

Interference agents or substances	Mechanism of interferences	Reference
Catheter and cannula	Through contaminating the device by i.v fluids (containing Na^+, K^+), i.v drug infusions or usage of high concentrations of clot activator.	Dimeski et al. (2010) Odum and Drenck (2002)
ISE membrane surfactants	Triton X-100 and Brij 35 which may be present in the calibrating, rinsing and quality control solutions; alter the selectivity and the membrane behavior. Benzalkonium surfactant that used to prevent thrombi forming, interacts with the K^+ valinomycin membrane temporarily to alter the membrane surface properties lead to false elevation of K^+ measurements by indirect ISE.	Lam et al. (2005) Malinowska and Meyerhoff (1998)
Therapeutic compounds (Ascorbic acid, Citrate and Mannitol)	Interfere by forming complexes with the ionophore, binding electrolytes of interest or changing the behavior of the membrane.	Meng et al. (2005)
Cell lysis	Specimen hemolysis or EDTA contamination are the most common cause of falsely rises in whole blood K^+.	Dimeski and Barnett (2005)
Anticoagulant contamination	Samples contaminated with potassium EDTA have an elevated K^+ and decrease the Ca^{2+} and Mg^{2+} by chelating.	Cornes et al. (2008)
Storage time and temperature	Storage temperature and time of storage of uncentrifuged blood have major influences on K^+ concentration by leaking out of cell K^+ content.	Sinclair et al. (2003) Trull et al. (2004)
Syringes	Syringes contain lithium heparin preparations caused false decreases in measured Ca^{2+} levels through binding of Ca^{2+} to heparin.	Biswas et al. (1981)
Collection tubes	Collection tubes containing anticoagulants and other additives such as silicone in the stopper lubricant was suggested as the causative agent of the interference in Li^+ and Mg^+ estimations by ISE system.	Sampson et al. (1997) Cao et al. (2001)
pH effects	Increased pH in the specimen lead to decrease both free Ca^{2+} and free Mg^{2+} concentrations indicating the stronger binding of these ions with proteins in the more alkaline environment.	Thode et al. (1990) Elin et al. (1996)

3 Objectives

ISE are used to measure some of the most critical analytes in clinical laboratories including blood gases, sodium, potassium, chloride, calcium and magnesium. Although the technology is regarded as sensitive and reliable; it has been shown to be not free of interferences in various studies in which blood samples from humans were used (Dimeski et al., 2010). As the research activities of the Clinic for Ruminants and Swine are directed to the disorders of potassium homeostasis in cattle, the present thesis focuses on the evaluation of different sources of influence on results obtained by direct ISE. To this end the aims of the present study are as follows:

1. To perform an agreement analysis between measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) on diluted plasma and undiluted whole blood samples of dairy cattle in order to find out if ISE (EML™ 105) could replace FAES (AAS, SOLAAR M6). In addition, the effects of hypo- as well as hyperproteinemia and hypercholesterolemia, as well as the impact of the extracellular pH-value on the results obtained by either of the two methods are investigated (**Trial I**).

2. To determine the extent to which the pre-analytical factors affect the outcome of the laboratory analysis, with respect to potassium and sodium ion concentrations. To this end, the influence of the time interval until further processing in the laboratory and the effect of different ambient temperatures on whole blood samples are evaluated (**Trial II**).

3. To investigate the effect of *in vitro* hemolysis on potassium ion concentrations in bovine plasma samples in order to establish a correction factor for falsely elevated potassium concentrations, using free plasma hemoglobin (FPHgb) as a measure of hemolysis (**Trial III**).

4. To obtain first insights into the relationship between the potassium content in skeletal muscle and potassium ion concentration in plasma (**Trial IV**).

4 Materials and methods

4.1 General information

4.1.1 Origin of blood samples and muscle biopsies

In the present study, blood samples were included that were sampled from healthy cattle owned by the Clinic for Ruminants and Swine, Freie Universität Berlin in the course of teaching units. These samples were obtained in agreement with the requirements of the committee for animal experimentation and were authorized by local authorities (LAGeSo, Berlin, Germany) under the registration number L0430/08. In addition, blood samples were included that were obtained during the routine blood sampling procedures from cattle that were referred to the animal hospital of the clinic or that originated from farm visits and were collected on a routine basis for metabolic profiling by members of the herd health service.

Cattle from which blood samples were obtained belonged to various breeds; most samples originated from cattle belonging to the breed German Holstein and - to a lesser extent - from the breeds Red Holstein and Galloway or from mixed breeds. On admission to the animal hospital, a complete clinical examination was performed (Rosenberger, 1990). Animal characteristics are given in the corresponding parts and in the Appendix (Tables 27, 29, 33 and 35).

Muscle biopsy samples (Trial IV) were collected from dead animals. The latter animals were patients of the University Hospital and had to be euthanized due to fatal prognosis of an internal or surgical disease. The samples were collected immediately following euthanasia.

4.1.2 Sampling

Blood sampling

Blood samples were drawn from the middle coccygeal vessels (tail blood vessels). The tail was raised to approximately 45^{o} with the horizontal line, the ventral surface of the tail was cleaned; the site of puncture is situated halfway between the two skin folds present at the basis of the tail.

A needle 18 or 21 gauge (depending on the animal size) was inserted into the blood vessel (Apple et al., 1982). The blood samples were collected into appropriate blood collecting tubes (Monovetten System, Sarstedt AG & Co., 51588 Nümbrecht, Germany) and analyzed within one hour following collection, a list of the type of samples, the sampling procedures and parameters examined is presented in Table 3.

Muscle biopsy technique is described in Chapter 4.5.5.

Table 3. An overview over the type of samples, sampling procedures, materials used and the analytes examined

Samples	Collection procedures	Analyte
Whole blood	Whole blood was collected using Monovette® 9 ml, Luer, Lithium-Heparin blood collection tubes (Nr. 02.265, 16 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany)	Potassium Sodium Glucose Lactate
	Whole blood was collected using Blutgas-Monovette®, Luer, 2 ml, Lithium-Heparin calcium-balanced (Nr. 05.1147.020, 50 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany)	pH pCO_2 HCO_3^- BE
Plasma	Plasma was harvested from the whole blood sample using Megafuge 3.0R centrifuge (Thermo Fisher Scientific GmbH, 63303 Dreieich, Germany)	Potassium Sodium Total protein Albumin Cholesterol Water mass content FPHgb
Muscle samples	Muscle samples collected by modified Bergström needle (Walter Veterinär-Instrumente e.K., 15837 Baruth,Germany)	Potassium Sodium

4.1.3 Sample processing

Blood samples were drawn into Monovette® 9 ml, Luer, Lithium-Heparin blood collection tubes (Nr. 02.265, 16 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany) to determine the levels of potassium, sodium, glucose and lactate. Blutgas-Monovette® tubes (Nr.

05.1147.020, 50 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany) were used to perform blood gas analysis.

Plasma samples were harvested after centrifugation of Li-heparinized whole blood samples using Megafuge 3.0R (Thermo Fisher Scientific GmbH, 63303 Dreieich, Germany) at 3000 rpm for 10 min at 20^{o}C. Plasma samples with evident hemolysis, visible as reddish discoloration of the plasma, were excluded. The plasma samples were kept in a plain tube until measurements.

4.2 Trial I, agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™ 105)

From in total 119 Li-heparinized whole blood samples collected from patients of the animal hospital for routine biochemistry analysis, 26 blood samples were selected for the agreement analysis between FAES (AAS, SOLAAR M6, Thermo Fisher Scientific, Cambridge, United Kingdom) and ISE (EML™105, Radiometer Medical A/S, Copenhagen, Denmark). The latter samples had normal levels of plasma water content, total protein, albumin, cholesterol and the results of blood gas analysis were within the reference range. The selection procedure was applied to avoid any interference caused by these analytes when sited outside the reference range. The reference ranges for the different analytes in whole blood and plasma are given in Table 4. The process was followed as described in the "Consensus on Standardization of Sodium and Potassium Measurements by ISE and FAES" established by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) for measurements of potassium and sodium by FAES in diluted plasma and ISE in whole blood samples (Burnett et al., 2000, CLSI, 2000).

From the remaining 93 samples, equal numbers of blood samples were selected with protein and cholesterol concentrations as well as pH-values less than, higher than and within the given reference range (Table 4), to investigate the effect of these analytes on the results of potassium and sodium measurements obtained by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) in diluted plasma samples and whole blood, respectively. Hemolyzed samples were excluded. The list of samples, parameters measured, methods adapted and the devices used are listed in Table 5.

Table 4. List of reference ranges for analytes in bovine blood samples used for inclusion of samples in the agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™105)

Analyte	Sample	Mean (Min.-Max.)	Source
pH-value	Whole blood	7.40-7.46	Laboratory of the Ruminant and Swine Clinic
pCO_2 (mmHg)		35-53	
HCO_3^-(mmol/l)		22-28	
Total protein (g/l)	Plasma	66-93	Scil Animal Care Company GmbH
Globulin (g/l)		45-55	
Albumin (g/l)		30-38	(Bertoni and Trevisi, 2013)
Cholesterol (mmol/l)		2-4.5	(Christinaz and Schatzmann, 1972)
Water mass (%)		93	(Rosenberger, 1990)
Potassium (mmol/l)		4.4 (4-5)	
Sodium (mmol/l)		145 (135-155)	

Table 5. An overview over analytes, analytical methods, instruments and sample materials (Trial I)

Analyte	Methods and Instruments		Sample Material
Sodium Potassium	Flame atomic emission method, AAS, SOLAAR M6 (Thermo Fisher Scientific, Cambridge, United Kingdom)		Plasma
	Ion selective electrode (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark)		Whole blood
pH pCO_2 BE HCO_3^-	Blood Gas Analyzer, ABL5™ (Radiometer Medical A/S, Copenhagen, Denmark)		Whole blood
Total protein	Biuret end point test kit	Automatic analyzer Cobas Mira (Cobas Mira Plus, Roche, Basel, Switzerland)	Plasma
Albumin	Bromocresol green test kit		
Cholesterol	cholesterol oxidase, phenol and aminophenazone test kit		
Water mass	Gravimetric method, Oven (AP 386, Memmert GmbH + Co.KG, Schwabach, Germany)		Plasma

4.2.1 Standardization of ISE (EML™ 105) and FAES (AAS, SOLAAR M6)

Two bovine serum pools with known values and a high degree of accuracy (Randox, Lot No. 415SN and 328SE, Randox Laboratories Ltd., Co. Antrim, BT294QY, United Kingdom, Figure 2) were used for standardizing ISE (EML™105) and FAES (AAS, SOLAAR M6)with respect to potassium and sodium measurements. The concentrations of total sodium and total potassium of

these pools are 138 mmol/l and 4.41 mmol/l, respectively, in quality control material level 2, and 153 mmol/l and 6.32 mmol/l, respectively, in quality control material level 3. Each of the two serum pools were analyzed in random order by alternating determinations on the ISE (EML™105) and FAES (AAS, SOLAAR M6), for a total of 15 replicates on each instrument for each concentration (CLSI, 2000). Bias of the ISE analyzer to flame photometry after the standardization process was fixed to be within ±2%.

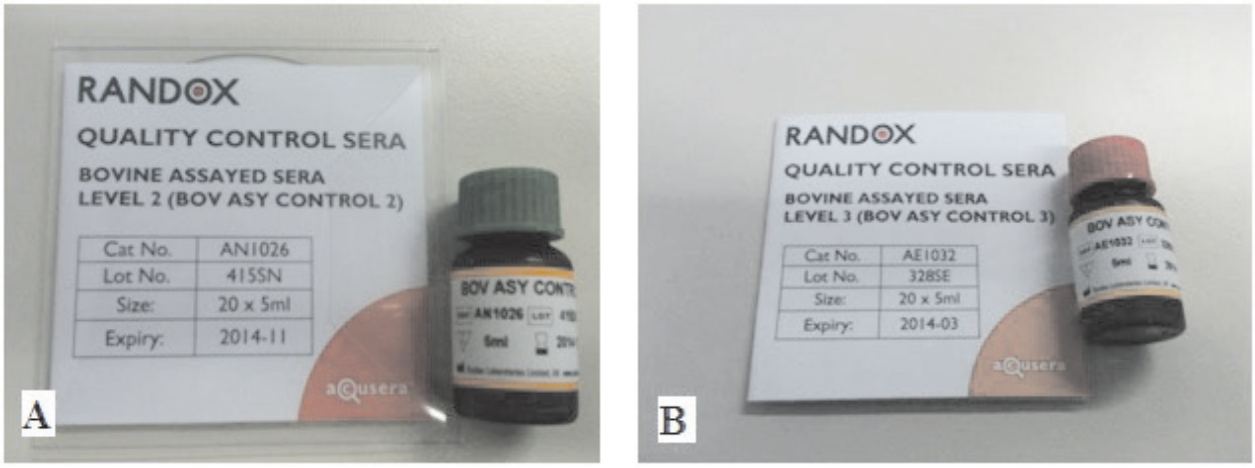

Figure 2. A comprehensive quality control bovine assayed serum (A. level 2 and B. level 3) designed for use in the routine monitoring of accuracy and precision in veterinary laboratories for commonly used veterinary parameters

4.2.2 Measurement of total potassium and sodium concentration by FAES (AAS, SOLAAR M6)

Measurements of total potassium and sodium concentrations were performed on diluted plasma samples using flame atomic emission spectroscopy technique carried out by AAS, SOLAAR M6 (Thermo Fisher Scientific, Cambridge, United Kingdom, Figure 3) at the laboratory of the clinic. In short the procedures are as follows: The sample is introduced into the flame as a sprayed solution. The heat from the flame evaporates the solvent and breaks chemical bounds to create free atoms; in addition, the flame provides thermal energy to the element leading to the excitement of valence electrons. When returning to basic state they emit energy as photons of a particular wave spectrum characteristic for that element. The intensity of light is directly proportional to the number of photons being emitted, which in turn is directly proportional to the

number of atoms in the solution. To prevent the ionization of samples due to high thermal energy, we used buffer solution which contains an element with lower ionization energy than that of analytes (Cesium in Schinkel buffer solution); the main wavelengths are used according to the manufacturer's manual.

4.2.2.1 Calibration of FAES (AAS, SOLAAR M6)

Flame atomic emission spectroscopy was calibrated using the three standards in aqueous solution. Potassium and sodium master standards and buffer solutions were used for preparation of the three standard solutions (Tables 6 and 7).

4.2.2.2 Measurement of samples

The measurements of total potassium and sodium concentrations were performed after the calibration process with the three standard solutions as follows; **Potassium plasma emission:** The plasma samples and the control materials were diluted separately to 1:40 with buffer solution, i.e. 250 µl of plasma or control materials + 9.75 ml buffer solution in 13 ml polystyrol tube (Nr. 55468001, Sarstedt AG & Co., 51588 Nümbrecht, Germany); then potassium was determined by flame atomic emission spectroscopy using an air-acetylene flame. The exposure surface of slot burner is 10 cm and with rotating the slot burner to 30^{o}, the sensitivity will be reduced and the linearity of the flame is improved, then the measurement was performed at wavelength 766.5 nm. **Sodium plasma emission:** The diluted plasma samples and control materials were diluted from potassium analysis 1:10 again with buffer solution to achieve final dilution of 1:400 then sodium was determined by flame atomic emission spectroscopy using an air-acetylene flame. The exposure surface of slot burner is 10 cm and with rotating the slot burner to 90^{o}, the sensitivity will be reduced and the linearity of the flame is improved, then the measurement was performed at wavelength 589 nm.

Figure 3. Flame atomic absorption / emission spectroscopy, (AAS, SOLAAR M6, Thermo Fisher Scientific, Cambridge, United Kingdom) used in emission mode for measuring total potassium and sodium concentrations in diluted bovine plasma samples

Table 6. Concentrations and composition of potassium and sodium master standards and buffer solution used for the calibration process of FAES (AAS, SOLAAR M6)

Reagent	**Concentration**	**Composition**
Potassium master standard	Potassium (10 mmol/l)	A 39.1 ml of potassium standard 1 g/l (Nr. 1AA-219, Ultra scientific, USA, obtained by LGC Standards GmbH, 46485 Wesel, Germany) was dissolved with 60. 9 ml bi-distilled water in a volumetric flask. Then the solution was stored in a plastic bottle.
Sodium master standard	Sodium (20 mmol/l)	A 46 ml of sodium standard 1 g/l (Nr. 1AA-211, Ultra scientific, USA, obtained by LGC Standards GmbH, 46485 Wesel, Germany) was dissolved with 54 ml bi-distilled water in a volumetric flask.
Buffer solution	---	The buffer solution used is cesium chloride-lanthanum chloride buffer solution (Schinkel solution Nr. 1.16755, Merck KGaA, 64293 Darmstadt, Germany). 50 ml of schinkel solution was diluted with 950 ml bi-distilled water to get the working buffer solution.

Table 7. Composition and dilution of standards used in the process of calibration for FAES (AAS, SOLAAR M6)

Standard	Dilution	Composition
Standard 3	1:40	25 ml of potassium master standard + 25 ml sodium master standard were dissolved in Schinkel buffer working solution added up to 1 liter
Standard 2	1:80	Standard 3 was diluted with Schinkel buffer working solution in a ratio 1:1
Standard 1	1:160	Standard 3 was diluted with Schinkel buffer working solution in a ratio 1:3

4.2.3 Measurements of potassium and sodium ion concentration by direct ISE (EML™ 105)

The direct ISE method using EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark (Figure 4) was applied for measuring potassium and sodium ions in whole blood samples. The procedures were performed according to the manufacturer's instructions.

4.2.3.1 Calibration of ISE (EML™ 105)

The standards used to calibrate the EML™ 105 analyzer were those provided by the manufacturer following the general methods used for calibration of ion selective electrodes at a constant ionic strength of 160mmol/l (Mohan and Bates, 1975). The two calibrating solutions used by the manufacturer: calibrating solution 1 (with values cK^+ = 4.0 mmol/l, cNa^+ = 145 mmol/l, cCa^{2+} = 1.25 mmol/l and cCl^- =106.24 mmol/l) and a calibrating solution 2 (with values cK^+ = 40 mmol/l, cNa^+ = 20 mmol/l, cCa^{2+} = 5.0 mmol/l and cCl^- = 52.70 mmol/l).

4.2.3.2 Measurement of samples

Heparinized whole blood samples from Monovette tubes were introduced by injection mode into the instrument. ISE were calibrated automatically using appropriate solutions obtained from the manufacturer in regular intervals during the day. Ion selective electrode (EML™ 105) converts the activity of specific ions dissolved in a solution into an electric potential, which can be

measured by a voltmeter. The potential is measured against a reference electrode which has stabile and known electrode potential, independent of the composition of the sample. Due to the great importance of accurate measurement of electrolytes by ISE, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) published "Recommendations for Measurement and Conventions for Reporting Sodium and Potassium by Ion Selective Electrodes in Undiluted Serum, Plasma or Whole Blood" (Burnett et al., 2000).

A convention is proposed whereby for routine clinical purposes results of ISE measurements of sodium and potassium in undiluted plasma should be reported in terms of substance concentration (mmol/l). Following the manufacturer's manual RADIOMETER has determined that the EML100/105 results for the measurement of potassium and sodium on normal whole blood (human) should be in accordance with the results obtained by flame photometry. To this end the measured electrolyte values are then corrected for systemic deviations from the reference methods according to the Nernst equation. The response of the electrode activity measurement is converted to a mmol reading by means of a constant proportionality factor in the analyzer as follows:

$$c\mathrm{K}^+ \text{ corrected} = \mathrm{K}_1 \times c\mathrm{K}^+ \text{ measured} + \mathrm{K}_2$$

$$c\mathrm{Na}^+ \text{ corrected} = \mathrm{K}_3 \times c\mathrm{Na}^+ \text{ measured} + \mathrm{K}_4$$

where K_1, K_2, $K_3$and K_4 are constant factors for human blood

$K_1 = 0.99520069$ $K_3 = 0.97521033$

$K_2 = -0.0609701$ $K_4 = 1.18653527$

Figure 4. Ion selective electrode (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark) applied for measurements of potassium and sodium ion concentrations in undiluted bovine whole blood samples

4.2.4 Blood gas analysis

Blood gas analysis was performed on heparinized whole blood samples. Blood samples were drawn from the middle coccygeal vessels (see 4.1.2) into Blutgas-Monovette® tube (Nr. 05.1147.020, 50 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany) that were capped rapidly after collection. Samples were analyzed within 60 minutes following collection. Air bubbles were collected by rotating the tube between hands and removed immediately with the first drop of the blood.

The blood gas analysis was performed using ABL5™ Blood Gas Analyzer (Radiometer Medical A/S, Copenhagen, Denmark) following the manufacturer's instructions. The required sample volume was 85 µl. The procedure is as follows: the analyzer aspirates the blood into a measuring chamber which is fitted with Ion Selective Electrodes. The pH electrode compares a potential developed at the electrode tip with a reference potential, the resulting voltage is proportional to the concentration of hydrogen ions. The pCO_2 electrode is a pH electrode with a Teflon or

silicone rubber CO_2 semi permeable membrane covering the tip. CO_2 combines with H_2O in the space between the membrane and the electrode tip to produce free hydrogen ions in proportion to the partial pressure of CO_2. The voltmeter, although actually measuring hydrogen ions, is calibrated in pCO_2. For pO_2, oxygen permeates a polypropylene membrane and reacts chemically with a phosphate buffer. The O_2 combines with water in the buffer, producing current in proportion to the number of oxygen molecules. The current is measured and expressed as partial pressure of oxygen. Bicarbonate and Base Excess levels are calculated automatically by the machine as derived parameters selected by the operator during the machine setup. After measurement the blood is automatically expelled into a waste container and the sample path is cleaned, ready for the next sample. Measurements of blood gases were corrected for body temperature and hemoglobin contents. The analysis results appeared on the screen and were printed on paper.

The instrument must be calibrated before each measurement and the calibration processes is automatically performed with two calibration solutions (Cal 1 and Cal 2) and two gas mixtures, Cal 1 (Nr. 943837 calibrating solution 1, pH: 7.383, Radiometer Medical A/S, Akandevej 21, 2700 Bronskoj, Denmark) runs every half hour and Cal 2 (Nr. 943839 calibrating solution 2, pH: 6.841, Radiometer Medical A/S, Akandevej 21, 2700 Bronskoj, Denmark) every 4 hours.

4.2.5 Determination of total protein, albumin, globulin and cholesterol

Automatic analyzer Cobas Mira (Cobas Mira Plus, Roche, Basel, Switzerland) was used for measurement of total protein, albumin and cholesterol concentrations in plasma samples.

The Biuret reaction was used to determine total protein contents in plasma samples using a test kit (test kit Nr. LT-TP 0253, Labor+ Technik, Robert-W.-Kempner-Str. 6, D-14167 Berlin) at 550 nm, albumin was measured by Bromocresol green reaction (test kit Nr. AB 362, Randox Laboratories Ltd., 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom) 600 nm, and cholesterol was measured by cholesterol oxidase, phenol and aminophenazone reaction (test kit Nr. LT-CH 0023, Labor and Technik, Robert-W.-Kempner-Str. 6, D-14167 Berlin) at 550 nm. Plasma globulin levels were calculated by subtracting the albumin from the total protein concentrations (Dimeski and Barnett, 2005).

4.2.6 Determination of water mass concentration

A gravimetric method (Oven-drying method) was chosen for determination of the plasma water content due to its high precision (de Jong et al., 1987). The measurement was performed on plasma samples. Glass test tubes were used for measuring plasma water mass concentration by determining its weight by Sartorius balance scale (Sartorius AG, 37075 Göttingen, Germany) (tube weight). The tubes were filled with 300 µl plasma at room temperature and weighed (wet weight), and then the tubes were heated at 110^{o}C in an oven (AP 386, Memmert GmbH + Co.KG, Schwabach, Germany) for at least 16 hours. After that, the tubes were sealed with plastic stoppers immediately after heating and left for 1-4 hours in desiccators for cooling, then the stoppers were removed for a moment to eliminate the vacuum caused by cooling then closed again to be weighted (dry weight).

The water content in % was calculated after Hatashita and Hoff (1990) as [(wet – dry weight) / wet weight] x 100.

4.2.7 Statistical analysis

Data were collected and managed by a Microsoft® Access Database 2007 file. The results were tested for normal distribution using Shapiro Wilks W Test and were found normally distributed. The level of significance was set at $p<0.05$.

For the agreement study, Bland and Altman method was performed using Prism 5.01 software (Graph Pad Software Inc., San Diego, California). The bias and variability of differences in the potassium and sodium measurements obtained by the two analyzers, ISE (EML™105) and FAES (AAS, SOLAAR M6) were measured. The difference between the two measurements was calculated and plotted against the mean of the two measurements according to (Bland and Altman, 1999). Then the 95% limits of agreement were calculated as (d±1.96xSD), where d is the mean difference and SD is the standard deviation.

For investigating the effect of values outside the reference range including plasma total protein, albumin, globulin, cholesterol and pH-values on potassium and sodium measurements by ISE

(EML™105) and FAES (AAS, SOLAAR M6), the data were analyzed statistically using SPSS Statistics® 17.0 (Version 17.0 released 2008, SPSS Inc., Chicago). Pearson's correlation was used to estimate the relationship between the concentrations of these parameters and the differences between the two methods. The results are plotted with the differences of the two methods presented on the Y-axis against the concentrations of total protein, albumin, globulin, cholesterol and the pH-values on the X-axis. The correlation coefficients were interpreted using the scale provided by Salkind, where the values between (0.8 and 1.0), (0.6 and 0.8), (0.4 and 0.6), (0.2 and 0.4) and (0.0 and 0.2) were defined as very strong, strong, moderate, weak and very weak or no relationship, respectively (Chung, 2007). The level of significance was set at $p<0.05$ Table 12.

4.3 Trial II, pre-analytical factors (effect of storage duration and temperature)

The concentrations of sodium, potassium, glucose, lactate and blood gases were determined in whole blood samples and measurements were repeated in intervals starting from time point zero (baseline measurement) and subsequent measurements in intervals until 72 hours. Samples were stored either cooled at 4°C or at room temperature (22°C) until the measurement.

4.3.1 Animals

Blood samples from a total of ten female German Holstein cows admitted to the Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin suffering from various disorders were included in this study. The basic animal characteristics are listed in Table 33 in the Appendix.

4.3.2 Blood samples and sampling procedures

Heparinized whole blood samples were collected from each animal in three separate Monovette® 9 ml, Luer, Lithium-Heparin blood collection tubes (Nr. 02.265, 16 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany). The blood samples were collected at the time of admission or few days after the admission. Samples were taken from the middle coccygeal vessels (see chapter 4.1.2). The syringes were filled one after another and numerated according to the order of collection. The samples were divided into two aliquots. One stored at 4°C and the

second at 22°C until measurements took place. All samples were measured following sampling (baseline) and subsequently in intervals up to 72 hours (0min, 20min, 35min, 50min, 65min, 80min, 95min, 24h, 48h and 72h after collection), the method modified after Brito et al. (2008).

4.3.3 Measurement of potassium and sodium ions concentration

Potassium and sodium ion concentrations, glucose levels and blood lactate were measured in heparinized whole blood samples by using ion selective electrode device (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark) (Table 8) Measurements were performed according to the manufacturer's instructions (see chapter 4.2.3).

4.3.4 Blood gas analysis

Measurements of pH, pCO_2, HCO_3^- and Base Excess were performed on heparinized whole blood samples using Blood Gas Analyzer (ABL5™, Radiometer Medical A/S, Copenhagen, Denmark) (Table 8), the sample was collected using Blutgas-Monovette® tube (Nr. 05.1147.020, 50 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany). The method was performed according to the manufacturer's instructions. The sample was analyzed immediately and the results were corrected for body temperature and hemoglobin content in the sample. Detailed explanation of the blood gas analysis is listed in chapter 4.2.4.

Table 8. List of analytes, sample types, methods and devices used for investigating the effect of pre-analytical factors (storage duration and temperature) on the results of potassium and sodium measurements by ISE (Trial II)

Parameter	Methods and devices	Samples
Potassium Sodium Glucose Lactate	Ion selective electrode (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark)	Whole blood
Blood gases: pH pCO_2 BE HCO_3^-	Blood Gas Analyzer, ABL5™ (Radiometer Medical A/S, Copenhagen, Denmark)	Whole blood

4.3.5 Statistical analysis

Data were collected and managed by a Microsoft® Access Database 2007 file and analyzed by using Prism 5.01 software (Graph Pad Software Inc., San Diego, California). Kruskal-Wallis test was used for comparing the values of pH, pCO_2, HCO_3^-, Base Excess and the concentration of sodium, potassium, glucose and blood lactate, in relation to the baseline measurements and these measurements in intervals until 72 hours at 4°C and 22°C. Spearman correlation was performed using SPSS Statistics® 17.0 (Version 17.0 released 2008, SPSS Inc., Chicago), to find out if the changes in potassium and sodium relate to the changes in the blood biochemical variables (pH, pCO_2, HCO_3^-, BE, glucose and lactate) in cattle blood samples stored at 22°C and 4°C with time interval up to 72 hours. The significance level was set at<0.05. The results were graphed using the mean and standard error (Brito et al., 2008) (Table 12).

4.4 Trial III, effect of *in vitro* hemolysis on potassium ion concentration

4.4.1 Animals

Blood samples from 20 cattle (Ten German Holstein and ten Galloway cattle) were included in the present study. The basic animal characteristics are listed in Table 34 in the Appendix.

4.4.2 Blood samples and sampling procedures

Whole blood samples were obtained during routine blood collection procedures for diagnostic reasons. Whole blood samples were collected from each animal using Monovette® 9 ml, Luer, Lithium-Heparin blood collection tubes (Nr. 02.265, 16 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany) (see chapter 4.1.2). Samples were categorized into 4 groups as shown in Table 10, the design of this study was performed according to a previous study by Owens et al. (2005) who established a correction factor for factitiously elevated potassium using free plasma hemoglobin as a measure of *in vitro* hemolysis. List of parameters measured and devices used are listed in Table 9.

Table 9. List of analytes, type of sample and instruments used for investigating the effect of *in vitro* hemolysis on the results of potassium measurements in bovine blood samples **(**Trial III)

Parameter	Methods and devices	Samples
Potassium	Ion selective electrode (EML^{TM} 105, Radiometer Medical A/S, Copenhagen, Denmark)	Plasma
FPHgb	Spectrophotometer Harboe method, Visible spectrophotometer (LKB Novaspec II, Amersham Pharmacia biotech currently GE Healthcare Europe GmbH, 79111 Freiburg, Germany)	Plasma

Table 10. Overview over the different manipulations performed on bovine blood samples for induction of *in vitro* hemolysis (Trial III)

Group	Manipulation of samples
Group I	No manipulation.
Group II	Manual hemolysis by ejection (either single or multiple ejections) of the blood immediately after collection into a vacuum tube with narrow needle (23G); for obtaining different concentrations of FPHgb.
Group III	Potassium acetate (0.054 mol/l) was added to the samples in different concentrations (either 25µl/ml or 50µl/ml) to induce significant hyperkalemia *in vitro* without causing hemolysis to the samples. This was previously described and verified by Owens et al. (2005) who analyzed the plasma hemoglobin before and after its addition.
Group IV	Both manual hemolysis as described for group II in addition to adding potassium acetate described for group III.

4.4.3 Measurements of plasma potassium concentration

Ion selective electrode device (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark) was used for measuring potassium concentrations in plasma samples. The samples were introduced from plastic syringes by the injection mode into the machine. The procedures of measurement were performed according to the manufacturer's instructions (see chapter 4.2.3).

4.4.4 Measurements of free plasma hemoglobin levels (FPHgb)

Visible spectrophotometer (LKB Novaspec II, Amersham Pharmacia Biotech currently GE Healthcare Europe GmbH, Munzinger Str. 5, 79111 Freiburg, Germany) was used for the measurement of free plasma hemoglobin in plasma samples coupled with a voltmeter for accurate wavelength settings. The measurement of FPHgb was performed in hemolyzed samples (group II and IV). Calibration and validation of the instrument were performed according to the manufacturer's instructions. Harboe (1959) method was used for measuring plasma hemoglobin in which 100 µl of the plasma sample were diluted in 1000 µl of 10 mg/dl Na_2CO_3. Absorbance values were measured relative to a 10 mg/l Na_2CO_3. The measurements were performed at 3 points of the absorbance spectrum (380, 415, 450 nm) using the 415 nm relative hemoglobin absorbance peak. To convert the absorbance measurements to concentrations directly the following equation was used according to Harboe (1959):

$$Hb\ mg/dl = 167.2\ X\ A_{415} - 83.6\ X\ A_{380} - 83.6\ X\ A_{450}$$

4.4.5 Statistical analysis

Data were collected and managed in an Excel sheet and analyzed by using SPSS Statistics® 17.0 (Version 17.0 released 2008, SPSS Inc., Chicago). The dependent variable presented on Y-axis was defined as the difference in potassium concentration between the hemolyzed (groups II and IV) and non-hemolyzed specimens (groups I and III) and the X-axis represents the concentration of plasma hemoglobin. The obtained slope between the variables is the potassium correction factor within 95% confidence interval (Table 12).

4.5 Trial IV, the relationship between muscle and plasma potassium concentrations

4.5.1 Animals

A total of 13 German Holstein cattle, admitted to the Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin aged from one month to two years that suffered from various disorders, were included in the present study. The decision of including these

animals into the study was taken after the owner had given the permission for euthanasia due to fatal prognosis; the basic animal characteristic data are listed in Table 35 in the Appendix.

4.5.2 Blood samples and sampling procedures

One Li-heparinized whole blood sample was collected from the intravenous catheter, that was introduced into the jugular vein prior to administration of the barbiturate for euthanasia, into Monovette® 9 ml, Luer, Lithium-Heparin blood collection tubes (Nr. 02.265, 16 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany), followed by harvesting the plasma samples after the centrifugation at 3000 rpm for 10 min at 20^{o}C using Megafuge 3.0R (Thermo Fisher Scientific GmbH, 63303 Dreieich, Germany). The plasma samples were kept in a plain tube for measurement of potassium and sodium ion concentrations (see chapter 4.1.2).

Table 11. List of analytes, samples, methods performed and devices used for investigating the relationship between muscle and plasma potassium concentrations in cattle (Trial IV)

Parameter	Methods and devices	Samples
Potassium Sodium	Ion selective electrode (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark)	Plasma
Estimation of sodium and potassium concentrations in muscle biopsy samples	Atomic absorption spectroscopy (contrAA 700, Analytik Jena GmbH, Jena, Germany).	Muscle

4.5.3 Measurement of potassium and sodium in plasma

Ion selective electrode (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark) was used for measuring potassium and sodium concentrations in plasma samples. The samples used for measurements were introduced from plastic syringes by the injection mode into the machine. The procedures were performed according to the manufacturer's instructions (see chapter 4.2.3).

4.5.4 Blood gas analysis

Measurements of pH, pCO_2, HCO_3^- and Base Excess were performed using heparinized whole blood samples by Blood Gas Analyzer (ABL5™, Radiometer Medical A/S, Copenhagen,

Denmark). Samples were collected using Blutgas-Monovette®, Luer, 2 ml, Lithium-Heparin calcium-balanced (Nr. 05.1147.020, 50 I.U. Heparin/ml blood, Sarstedt AG & Co., 51588 Nümbrecht, Germany). Measurements were performed according to the manufacturer's instructions. The sample was analyzed immediately following sampling and the result was corrected for body temperature and hemoglobin content. A detailed explanation of the process of blood gas analysis is given in chapter 4.2.4.

4.5.5 Muscle biopsy

4.5.5.1 Sampling technique

Muscle biopsies for determination of intracellular sodium and potassium were obtained immediately after euthanasia from 13 animals. The samples were obtained from the vastus lateralis muscle using a modified Bergström needle (Walter Veterinär-Instrumente e.K., 15837 Baruth, Germany) (6 mm diameter, 100 mm length). The sampling technique was performed according to the method reported by Leitner (2009) and Bergström (1975). The muscle biopsy samples were collected immediately after euthanasia of study animals which was performed by intravenous administration of 60 mg/kg BW Pentobarbital preparation (Euthadorm®, 400mg/ml, CP -Pharma, Burgdorf, Germany). An area of approximately 15 cm x 15 cm was shaved and cleaned. A small incision of 1-1.5 cm length was performed including skin and fascia. The biopsy sample was obtained by use of the Bergström needle. Approximately 100-150 mg of biopsy material was sampled and all visible connective tissue and fat were quickly removed from the sample. Then the sample was transferred into a pre-weighed 5-ml dry plain tube within 1 min of sampling. Two biopsy specimens were collected, one sample was used for histological examination to exclude that, fat or connective tissue which may affect the measurements and the second sample stored at -24°C until further analysis. After collecting the required muscle biopsy samples, the samples were sent to the laboratory of the Institute of Animal Nutrition, Freie Universität Berlin, Germany for determination of sodium, potassium and water content of muscle tissue samples.

4.5.5.2 Measurement of potassium and sodium content in muscle biopsy samples

For determination of dry matter content (DM) the sample was weighed (E) and then weighed again in a crucible before ashing (T1), then the sample was ashed for 1 hour at 120°C and then dried for 3 hours at 103°C. After cooling down, the sample was weighed again (T2) and the dry matter content was calculated according to the formula:

$$DM\ (g/kg) = ((T1 - T2) / E) \times 1000]$$

In order to determine the inorganic residue (raw ash – RA), the organic components of the dried samples were weighed in a crucible (T1). After ashing the samples at 600°C in a muffle furnace they were cooled down and weighed again (T2).

$$RA\ (g/kg) = [(T1 - T2) / E] \times 1000$$

The crucible content was mixed with 2.1 ml concentrated muriatic acid (37–38%) and 7 ml distilled water. It was then kept at 210–220°C over 50 minutes and subsequently cooled down again. The content was poured through a filter into a volumetric flask and filled with distilled water up to the 25 ml mark. The content of sodium and potassium in the ash solution was determined by atomic absorption spectroscopy (contrAA 700, Analytik Jena GmbH, Jena, Germany). The electrolyte contents measured in dry skeletal muscle tissue were expressed as gram per kilogram of dry weight. Then the measured electrolytes in dry tissue were converted to mmol/kg.

4.5.6 Statistical analysis

Data were statistically analyzed and graphically presented using SPSS Statistics® 17.0 (Version 17.0 released 2008, SPSS Inc., Chicago). The data were tested for normal distribution using Shapiro Wilks W Test and were found normally distributed. The level of significance was set at $p<0.05$. Pearson correlation was used for estimating the relationship between the concentration of potassium and sodium in plasma and muscle tissue and the effect of pH-values on plasma and muscle potassium concentration (Table 12).

Table 12. Summary of statistical methods used in the present study

	Statistical method	Purpose
Trial I	Bland and Altman 1999 method	For agreement analysis between FAES and ISE
	Pearson's correlation	To estimate the relationship between total protein, albumin, globulin, cholesterol concentrations and pH-values and the measurement of potassium and sodium by FAES and ISE
Trial II	Kruskal-Wallis test	To study the effect of storage duration and temperature on pH, pCO_2, HCO_3^-, Base Excess and the concentration of sodium, potassium, glucose and blood lactate
	Spearman correlation	To find out if the changes in potassium and sodium relate to the changes in pH, pCO_2, HCO_3^-, BE, glucose and lactate
Trial III	Plotting difference in potassium concentration (hemolyzed minus non-hemolyzed) on Y-axis against the concentration of plasma hemoglobin (X-axis)	For obtaining a correction factor for potassium concentration in hemolyzed samples by the slope between the variables
Trial IV	Pearson correlation	To find the relationship between muscle and plasma potassium concentrations

5 Results

5.1 Trial I, agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™ 105)

In total 119 blood samples were included that were obtained during routine blood sampling procedures at the Clinic for Ruminants, Faculty of Veterinary Medicine, Freie Universität Berlin for agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™ 105) for potassium and sodium measurements. In addition, the effect of high and low levels of total protein, albumin, globulin and cholesterol as well as the pH-value on potassium and sodium measurements by the two analyzers was evaluated.

5.1.1 Results of standardization process

The results of the standardization process performed on 15 replicates of two bovine serum pools [level 2 “sodium 138 mmol/l and potassium 4.41 mmol/l” and level 3 “sodium 153 mmol/l and potassium 6.32 mmol/l”] showed that, the Coefficient of Variation (CV%) was found to range from 0 to 0.93% for potassium and sodium (ISE and FAES) and the mean differences (Bias) of the ISE (EML™ 105) to FAES (AAS, SOLAAR M6) for the level 2 and level 3 quality control were 0.06 mmol/l and -0.15 mmol/l for potassium and -0.57 mmol/l and -0.25 mmol/l for sodium (Table 13).

Table 13. Results of standardization process using 15 replicates of quality control materials (level 2 and level 3) analyzed by the two analyzers (FAES and ISE). The CV% within-run is ranged from 0 to 0.93% for potassium and sodium

Quality control		Potassium concentration (mmol/l)			Sodium concentration (mmol/l)		
		FAES	ISE	Mean bias	FAES	ISE	Mean bias
Level 2 (n=15)	Mean ± SD	4.56±0.04	4.5±0	0.06	139.16±1.29	139.73±0.44	-0.57
	CV%	0.82	0		0.93	0.32	
Level 3 (n=15)	Mean ± SD	6.34±0.05	6.49±0.03	-0.15	153.95±1.27	154.2±0.4	-0.25
	CV%	0.85	0.52		0.82	0.26	

5.1.2 Agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™ 105)

From in total 119 Li-heparinized whole blood samples collected from patients of the animal hospital for routine biochemistry analysis, 26 blood samples were considered for the agreement analysis between FAES (AAS, SOLAAR M6, Thermo Fisher Scientific, Cambridge, United Kingdom) and ISE (EML™105, Radiometer Medical A/S, Copenhagen, Denmark) with respect to potassium and sodium measurements. Samples with normal levels of plasma water content, total protein, albumin, cholesterol as well as normal pH-values and pCO_2 were selected to avoid any interference caused by these analytes when sited outside the reference range. The results of potassium or sodium measurements obtained from FAES (AAS, SOLAAR M6) in diluted plasma samples are expressed as the total concentrations and that obtained from ISE (EML™ 105) in undiluted whole blood samples are expressed as ionized potassium or sodium concentrations.

The mean values ± SD of potassium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) were 4.08±0.51 mmol/l and 4.13±0.50 mmol/l, respectively, and those of sodium were 136.51±3.10 mmol/l and 139.46±1.92 mmol/l, respectively (Table 14). The mean differences between the two methods were -0.04±0.11mmol/l for potassium (Bias 0.96%) and -2.9±2.6 mmol/l, for sodium (Bias 2%), respectively (Table 15).

Figures 5 and 6 illustrate the results of total potassium and sodium concentrations measured by FAES (AAS, SOLAAR M6) plotted against potassium and sodium concentrations obtained by ISE (EML™ 105), the graph shows the line of equality which makes it easier to assess visually how well the two methods agree.

The average values of the measurements obtained by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) are plotted against their differences for each sample using Bland and Altman method. The plot also shows the extremes or outlier values (Figures 7 and 8). The 95% limits of agreement for potassium values estimated by mean difference ± 1.96 SD of the differences between the two methods were found to lie between 0.17 to -0.26 mmol/l (Table 15 and **Figure 7**) and that for sodium were found to lie between 2.2 to -8.11 mmol/l (Table 15 and Figure 8).

Table 14. Agreement analysis between FAES and ISE for analysis of potassium and sodium in plasma and whole blood samples, respectively, from cattle. The table shows mean values± SD, minimum and maximum values of selected analytes in samples from 26 cattle

	n=	Min.	Max.	Mean ±SD
pH-values	26	7.37	7.47	7.41±0.02
pCO_2 (mmHg)	26	36.00	54.00	44.50±5.89
HCO_3^-(mmol/l)	26	21.00	33.00	27.15±3.47
Plasma total protein (g/l)	26	65.9	80.00	73.55±4.14
Plasma albumin (g/l)	26	30.40	38	35.55±2.1
Plasma globulin (g/l)	26	27.00	45.40	37.35±5.22
Plasma cholesterol (mmol/l)	26	1.90	4.63	2.57±0.71
Plasma water mass (%)	26	92.52	93.98	93.04±0.36
Total potassium concentration (mmol/l, FAES)	26	3.10	4.97	4.08±0.51
Ionized potassium concentration (mmol/l, ISE)	26	3.10	4.90	4.13±0.50
Total sodium concentration (mmol/l, FAES)	26	131.39	142.81	136.51±3.10
Ionized sodium concentration (mmol/l, ISE)	26	136.00	143.00	139.46±1.92

Table 15. Results of potassium and sodium measurements obtained by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) in 26 bovine diluted plasma and undiluted whole blood samples, respectively. The results are expressed as mean values ± SD

Methods	Sodium (mmol/l)	Potassium (mmol/l)
FAES	136.51±3.10	4.08±0.51
ISE	139.46±1.92	4.13±0.50
Mean differences (MD) ± SD	-2.9±2.6	-0.04±0.11
95% limits of agreement (MD ± 1.96 x SD)	2.2 to -8.11	0.17 to -0.26

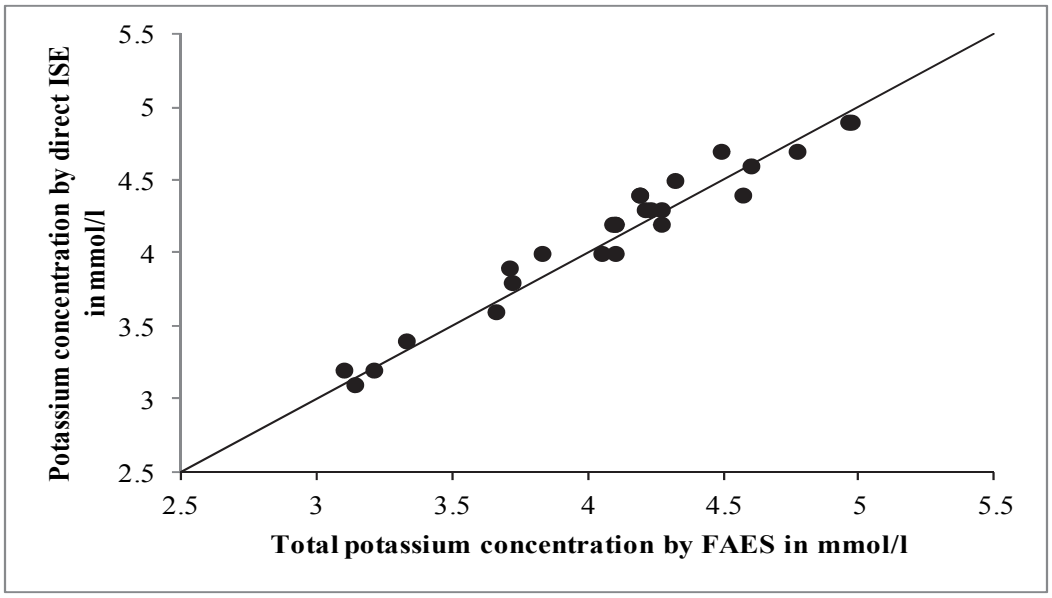

Figure 5. Total potassium concentrations in 26 diluted plasma samples from cattle as obtained by FAES (AAS, SOLAAR M6) and plotted against ionized potassium concentrations in whole blood samples obtained by ISE (EML™ 105). The graph illustrates the line of equality and the visual agreement between these two methods

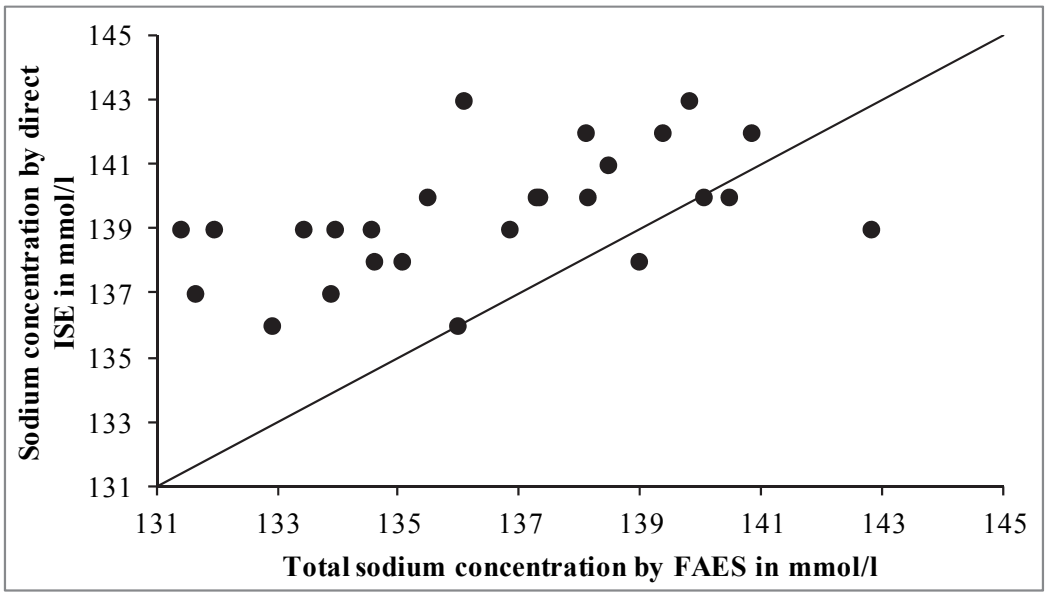

Figure 6. Total sodium concentrations in 26 diluted plasma samples from cattle obtained by FAES (AAS, SOLAAR M6) and plotted against ionized sodium concentrations in whole blood samples obtained by ISE (EML™ 105). The graph illustrates the line of equality and the visual agreement between these two methods

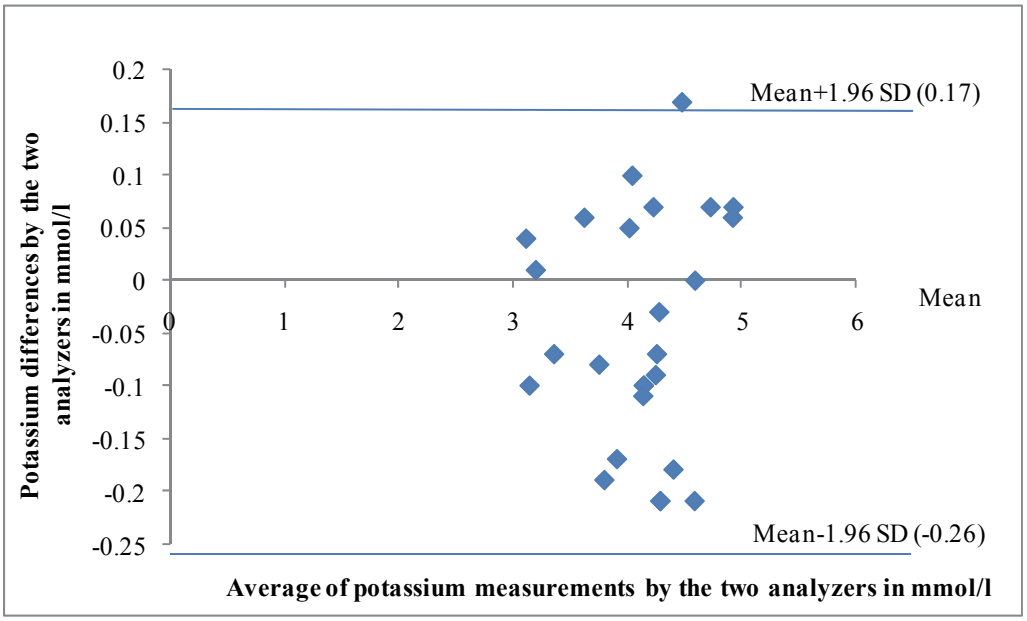

Figure 7. Agreement analysis for potassium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) in 26 diluted plasma and undiluted whole blood samples from cattle. The graph illustrates the comparison of the average of the results for potassium measurements as determined by the two analyzers against their differences. The 95% limits of agreement for potassium values estimated by mean difference ± 1.96 SD of the differences between the two methods were found to lie between 0.17 to -0.26 mmol/l

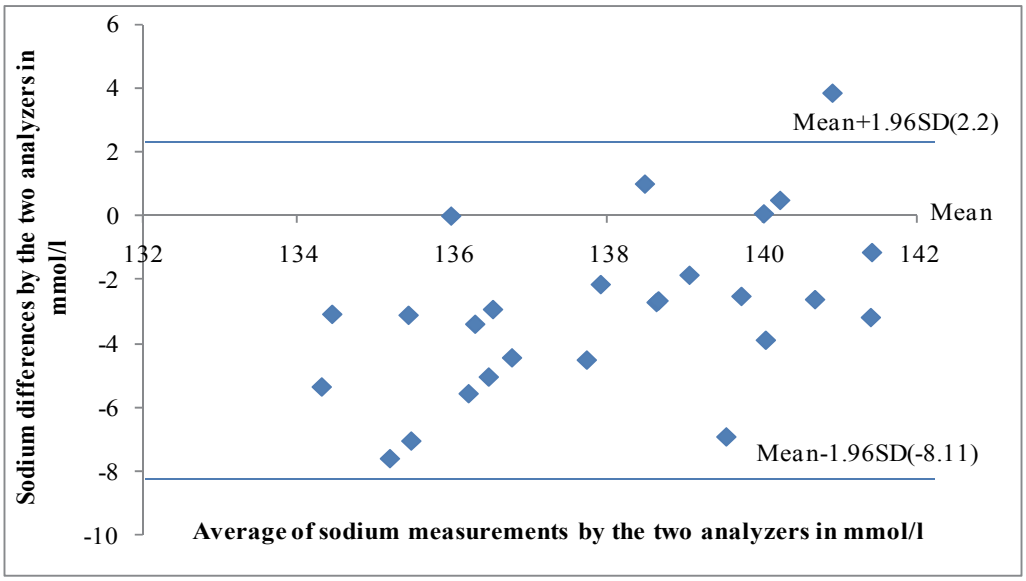

Figure 8. Agreement analysis for sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) in 26 diluted plasma and undiluted whole blood samples from cattle. The graph illustrates the comparison of the average of sodium measurements as determined by the two analyzers against their differences. The 95% limits of agreement for sodium values estimated by mean difference ± 1.96 SD of the differences between the two methods were found to lie between 2.2 to -8.11 mmol/l

5.1.3 Effects of total protein and cholesterol contents and pH on results of potassium and sodium measurements obtained by FAES (AAS, SOLAAR M6) and ISE (EML™ 105)

Blood samples from 93 German Holstein cattle with a mean age of 3.7 years (14 days 11 years) were included in this study. Samples were obtained during routine sampling procedures in the clinic. The animals suffered from various disorders including left displacement of the abomasum, right displacement of the abomasum, abomasal ulceration, secondary ketosis, mastitis, metritis, peritonitis, bronchitis and bronchopneumonia. Samples with variable concentrations of plasma total protein, cholesterol and pH-values (within and beyond the reference ranges) were selected. The detailed data of study animals and the laboratory findings are listed in the Appendix (Tables 29, 30, 31 and 32).

For each analyte, samples were allotted to one of three different groups (Group 1, values within the reference ranges given in Table 4, Group 2, values underneath the lower limit of the reference range, Group 3, values above the upper limit of the reference range) in order to investigate the effect of various levels of each analyte on the results of measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). The mean values, minimum and maximum of each group are listed in Table 16.

Table 16. Effect of total protein, albumin, globulin and cholesterol levels and pH on results of measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). The table shows mean values ± SD, minimum and maximum values for total protein, albumin, globulin and cholesterol concentrations and pH-values in blood samples from 93 cattle

	n=	Min.	Max.	Mean ± SD
Plasma total protein (g/l)	24	48.70	108.35	79.64±18.30
Plasma albumin (g/l)		26.00	39.70	33.66±3.49
Plasma globulin (g/l)		13.10	74.45	45.97±18.85
Plasma cholesterol (mmol/l)	39	0.01	6.97	3.11±2.09
pH-value	30	7.14	7.51	7.41±0.07

The effect of different levels of plasma total protein on potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) was studied by selecting 24 blood samples, 16 of which were outside the reference range; Group 1 (n = 8), samples within the reference range (66-93 g/l), Group 2 (n = 8) values underneath reference range; Group 3 (n = 8) samples above the reference range (Table 4). From the same samples, the effect of both albumin and globulin on potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) were investigated.

Three levels of plasma total protein as reflected in the three groups are plotted against the differences between the measurements of potassium and sodium by the two methods. There was a non-linear indirect modest correlation ($p<0.05$) between the change in plasma total protein concentration and the difference in potassium and sodium measurements by the two methods ($r = -0.44$ and -0.53, respectively) as shown in Tables 17, 18 and Figures 9 and 10. Low total protein levels tended to cause a pseudohyper- effect for both analytes, potassium and sodium, when FAES was applied whereas high total protein levels resulted in a pseudohypo- effect for samples when FAES was applied, whereby the effect was more prominent for sodium (Figures 9 and 10).

In the same 24 samples, the concentration of plasma albumin is plotted against the differences between the measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). No correlation was found between the changes in plasma albumin concentrations and the differences between the two methods for results of potassium and sodium measurements ($r = 0.1$ and 0.02, respectively, Figures 11 and 12). The concentration of plasma globulin is plotted against the differences between the measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105), demonstrating a non-linear indirect moderate correlation ($r = -0.44$ and -0.52, respectively, Figures 13 and 14). These findings demonstrate that globulins are more likely causing the pseudohypo- and pseudohyper- effect than albumin.

Table 17. Correlation between total protein, albumin and globulin content in bovine plasma as well as cholesterol concentrations and pH-values with the difference between the results obtained for measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). The results are expressed as r (p value), where r is the Pearson correlation coefficient. The asterisk * = significance at $p<0.05$ and ** = significance at $p<0.01$

	Differences between the results of potassium measurements by the two analyzers in mmol/l [r (p value)]	**Differences between the results of sodium measurements by the two analyzers in mmol/l [r (p value)]**
Plasma total protein (g/l)	-.44* (0.032)	-.53** (0.008)
Plasma albumin (g/l)	0.10 (0.658)	0.02 (0.929)
Plasma globulin (g/l)	-.44* (0.03)	-.52** (0.01)
Plasma cholesterol (mmol/l)	-0.22 (0.182)	-0.07 (0.674)
pH-value	0.18 (0.343)	0.22 (0.252)

Table 18. The difference range observed between FAES (AAS, SOLAAR M6) and ISE (EML™ 105) for results of potassium and sodium measurements in 24 diluted plasma and undiluted whole blood samples from cattle at different plasma total protein concentrations (within, below or above the limits of the reference range 66-93 g/l)

Difference range of potassium and sodium measurements (FAES - ISE) mmol/l at different levels of plasma total protein			
	Low total protein [n=8]	**Normal total protein [n=8]**	**High total protein [n=8]**
Potassium (mmol/l)	0.01 to 0.17	-0.31 to 0.03	-0.22 to 0.1
Sodium (mmol/l)	-6.91 to 5.2	-8.34 to 1.34	-12.11 to -1.62

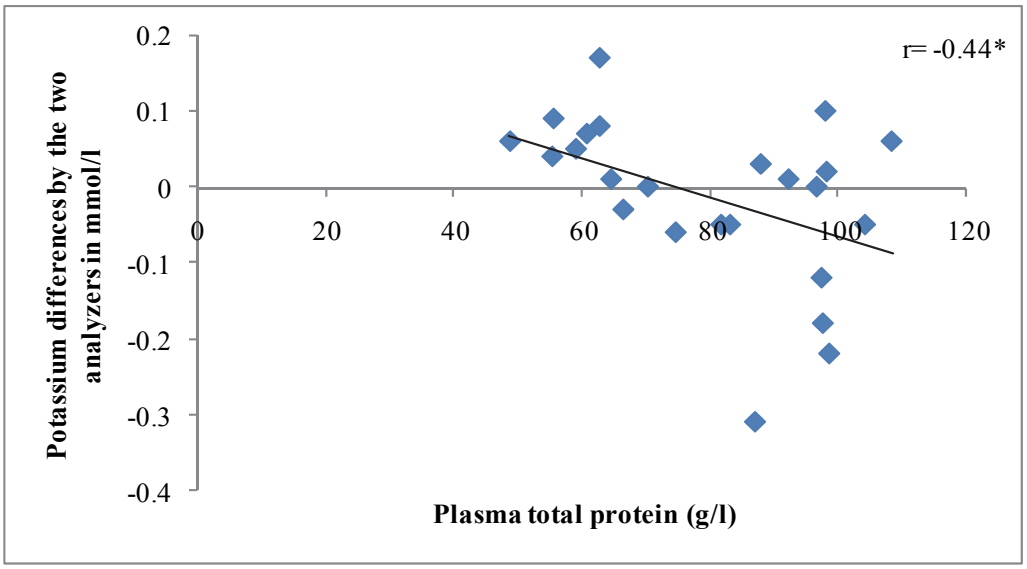

Figure 9. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™105) results for potassium measurements versus plasma total protein concentrations in 24 blood samples from cattle. The graph illustrates a non-linear indirect modest correlation (r=-0.44) between the change in plasma total protein concentration and the difference in potassium measurements by the two analyzers. The asterisk * = significance at $p<0.05$. r = Pearson correlation coefficient

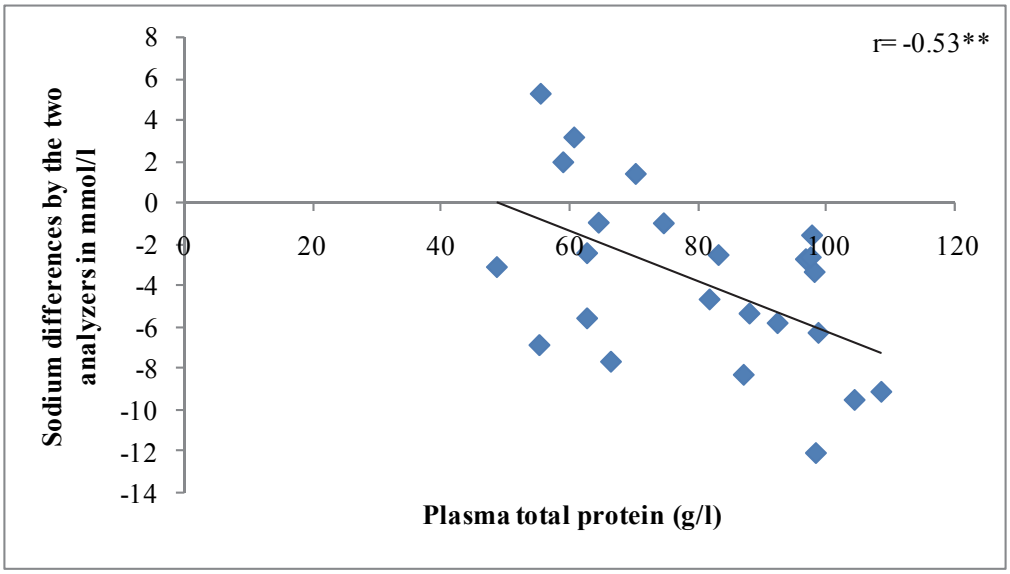

Figure 10. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™ 105) results for sodium measurements versus plasma total protein concentrations in 24 bovine plasma samples. The graph illustrates a non-linear indirect modest correlation (r=-0.53) between the change in plasma total protein concentration and the difference in sodium measurements by the two methods. The asterisk ** = significance at $p<0.01$. r = Pearson correlation coefficient

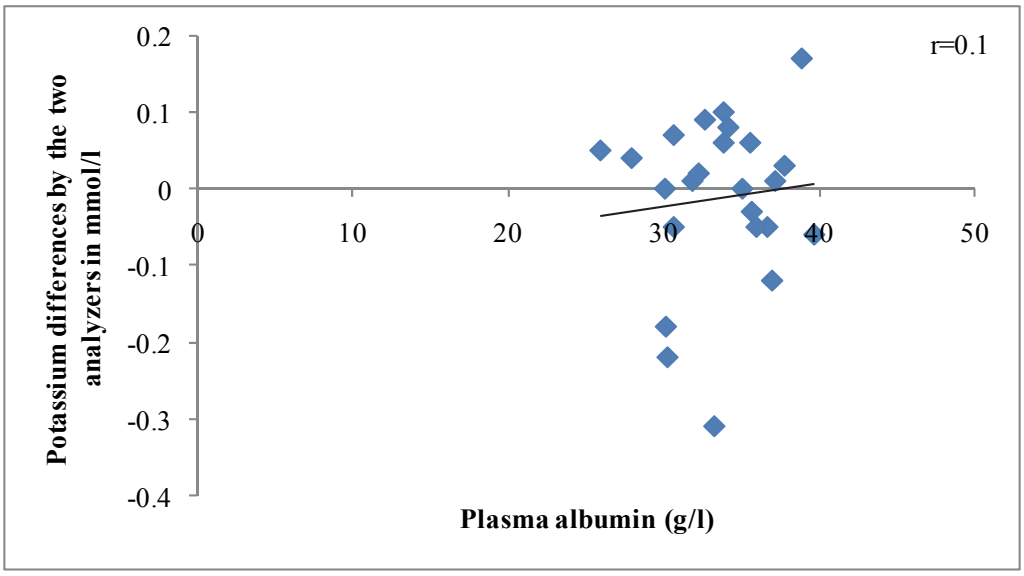

Figure 11. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™ 105) results for potassium measurements versus plasma albumin concentrations in 24 bovine plasma samples. The graph illustrates no correlation (r=0.1) between the change in plasma total protein concentration and the difference in potassium measurements by the two methods. r = Pearson correlation coefficient

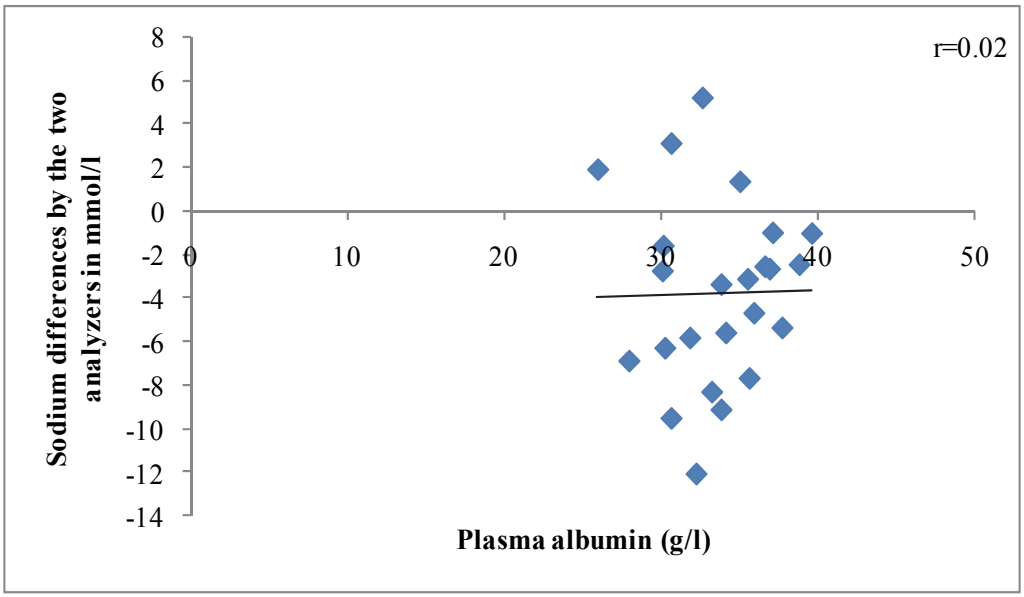

Figure 12. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™ 105) results for sodium measurements versus plasma albumin concentrations in 24 bovine plasma samples. The graph illustrates no correlation (r=0.02) between the change in plasma albumin concentrations and the difference in sodium measurements by the two methods. r = Pearson correlation coefficient

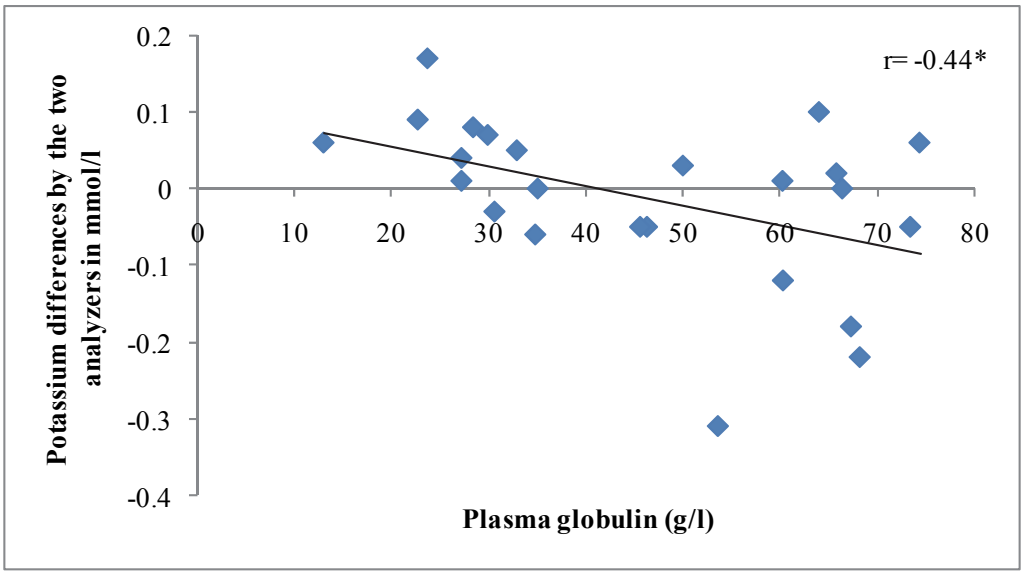

Figure 13. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™ 105) results for potassium measurements versus plasma globulin concentrations in 24 bovine plasma samples. The graph illustrates non-linear indirect moderate correlation (r=-0.44) between the change in plasma total protein concentration and the difference in potassium measurements by the two methods. The asterisk * = significance at $p<0.05$. r = Pearson correlation coefficient

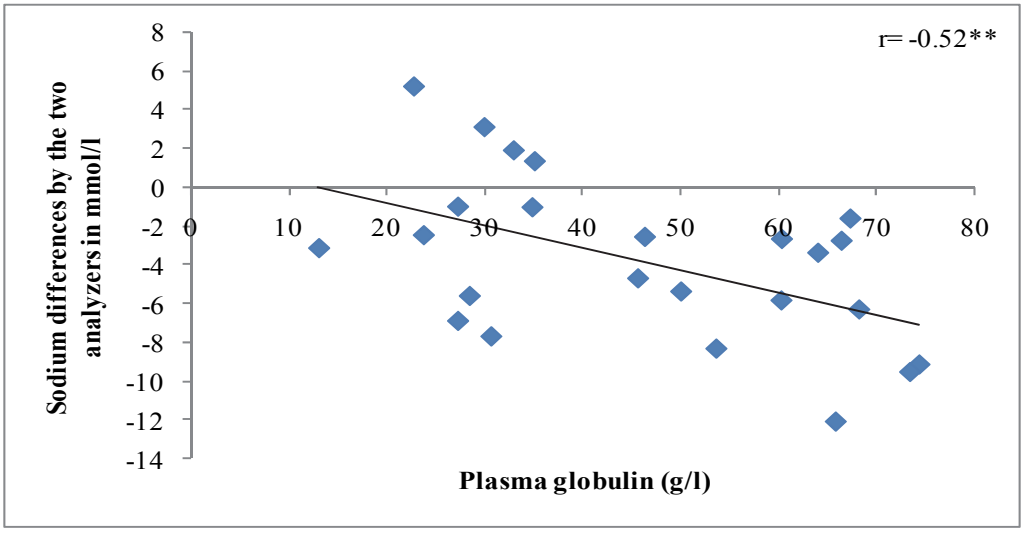

Figure 14. The relationship between the differences of FAES (AAS, SOLAAR M6)and ISE (EML™105) results for sodium measurements versus plasma globulin concentrations in 24 bovine plasma samples. The graph illustrates non-linear indirect moderate correlation (r=-0.52) between the change in plasma total protein concentration and the difference in sodium measurements by the two methods. The asterisk ** = significance at $p<0.01$. r = Pearson correlation coefficient

The effect of different plasma cholesterol concentrations on potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) was studied by selecting 39 blood samples, 26 of which were outside the reference range; Group 1 (n = 13), samples within the reference range (2-4.5 mmol/l), Group 2 (n = 13) values underneath reference range; Group 3 (n = 13) samples above the reference range (Tables 4 and 19). The concentrations of plasma cholesterol are plotted against the differences between the results of measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). Figures 15 and 16 illustrate non-linear indirect weak correlation with potassium and very weak correlation with sodium as r = -0.22 and -0.07, respectively.

The effect of different pH-values on potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) was studied by selecting 30 blood samples, 20 of which were outside the reference range; Group 1 (n = 10), samples within the reference range (7.40-7.46), Group 2 (n = 10) values underneath reference range; Group 3 (n = 10) samples above the reference range (Tables 4 and 20). The concentrations of pH-values are plotted against the differences between the measurements of potassium and sodium by the two methods. Figures 18 and 17 illustrate very weak and weak correlation between the change in plasma pH and the difference in potassium and sodium measurement by the two methods (r = 0.18 and 0.22, respectively).

Table 19. The difference range observed between FAES (AAS, SOLAAR M6) and ISE (EML™ 105) with respect to the results of potassium and sodium measurements in 39 diluted plasma and undiluted whole blood samples of cattle at different levels of plasma cholesterol (within, below or above the limits of the reference range 2-4.5 mmol/l)

Difference range of potassium and sodium measurements (FAES - ISE) mmol/l at different levels of plasma cholesterol			
	Low cholesterol [n=13]	**Normal cholesterol [n=13]**	**High cholesterol [n=13]**
Potassium (mmol/l)	0 to 0.32	-0.99 to 0.14	-0.41 to 0.25
Sodium (mmol/l)	-5.99 to 5.27	-12.77 to 1.71	-8.65 to 3.49

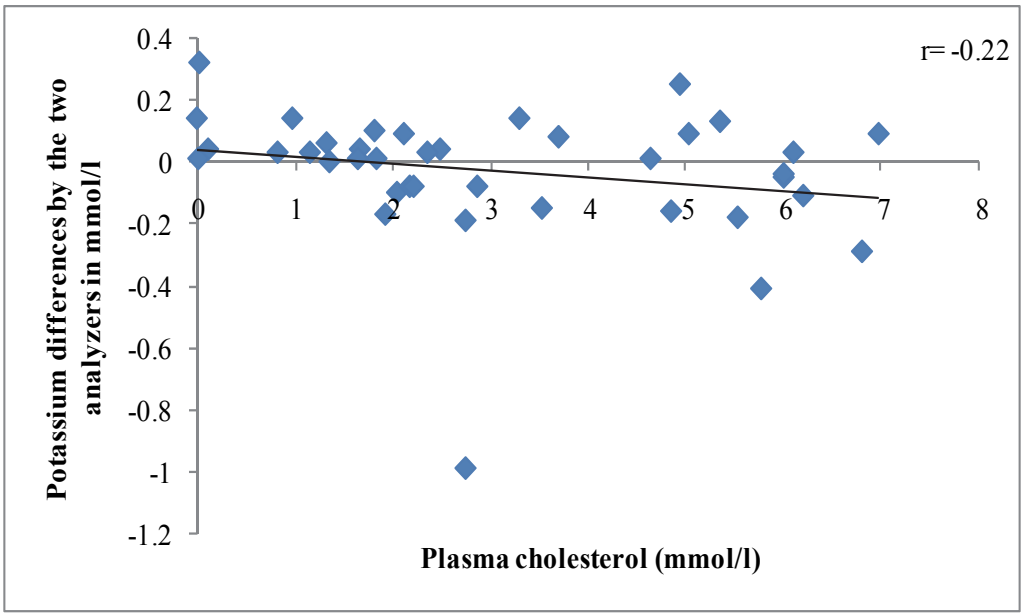

Figure 15. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™105) results for potassium measurements related to cholesterol concentrations in 39 plasma samples of cattle. The graph illustrates non-linear indirect weak correlation (r=-0.22) between the change in plasma cholesterol concentrations and the difference in potassium measurements by the two methods. r = Pearson correlation coefficient

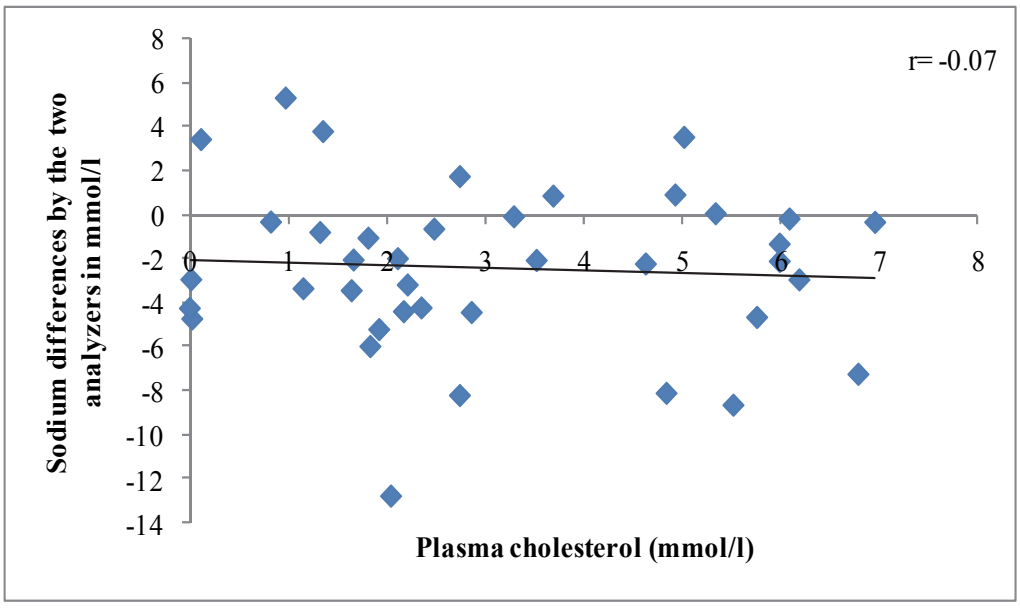

Figure 16. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™105) results for sodium measurements related to cholesterol concentrations in 39 plasma samples from cattle. The graph illustrates very weak correlation (r=-0.07) between the change in plasma cholesterol concentration and the difference in sodium measurements by the two methods. r = Pearson correlation coefficient

Table 20. The difference range observed between FAES (AAS, SOLAAR M6) and ISE (EML™ 105) with respect to results of potassium and sodium measurements in 30 diluted plasma and undiluted whole blood samples from cattle at different pH-values (within, below or above the limits of the reference range 7.40-7.46)

Difference range of potassium and sodium measurements (FAES - ISE) mmol/l at different levels of plasma pH-values			
	Low pH-values [n=10]	**Normal pH-values [n=10]**	**High pH-values [n=10]**
Potassium (mmol/l)	-0.24 to 0.03	-0.19 to 0.11	-0.29 to 0.09
Sodium (mmol/l)	-10.43 to -2.73	-8.61 to 7.79	-8.35 to 0.4

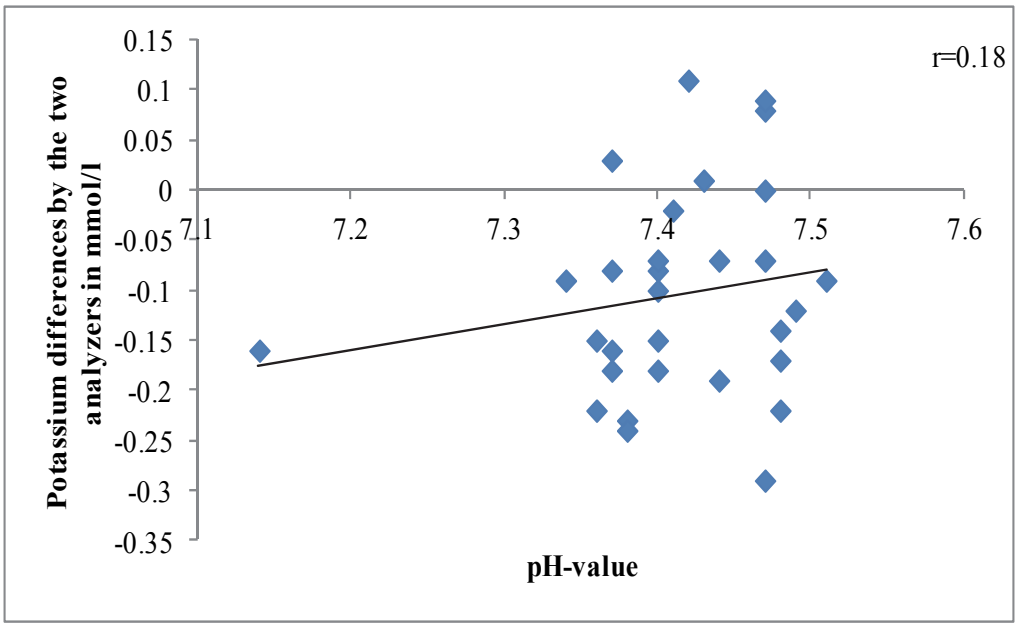

Figure 17. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™ 105) results for potassium measurements versus pH-values in 30 blood samples from cattle. The graph illustrates very weak correlation (r=0.18) between the change in pH and the difference in potassium measurements by the two methods. r = Pearson correlation

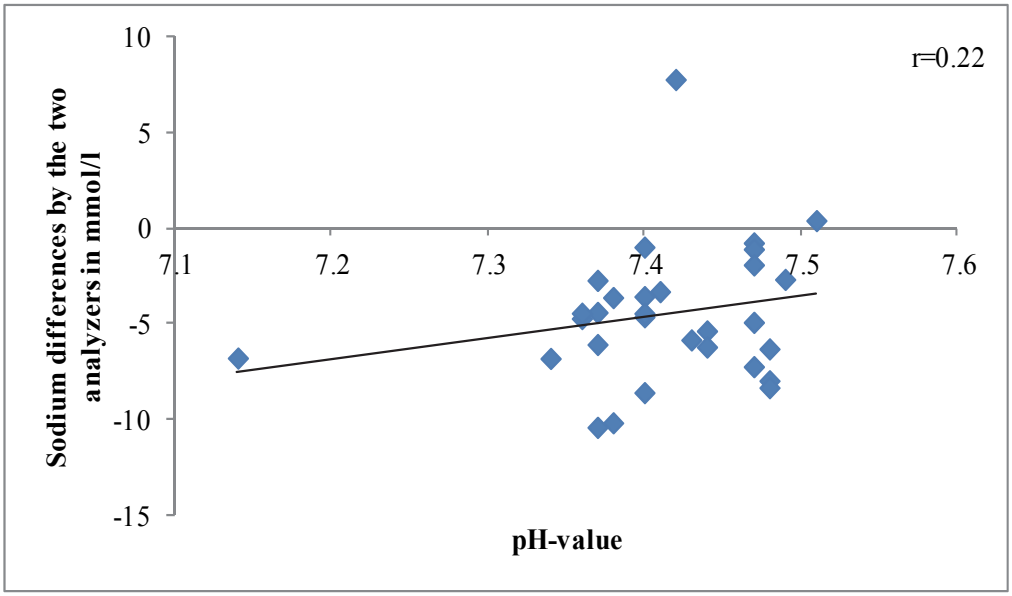

Figure 18. The relationship between the differences of FAES (AAS, SOLAAR M6) and ISE (EML™ 105) results for sodium measurements versus pH-values in 30 blood samples from cattle. The graph illustrates weak correlation (r=0.22) between the change in pH-values and the difference in results of sodium measurements by the two methods. r = Pearson correlation

5.2 Trial II, pre-analytical factors (effect of storage duration and temperature)

In this study, ten blood samples were harvested from ten dairy German Holstein cattle that were owned by or admitted to the Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin with various disorders. The animals had a mean age of 5.87 years (2.5 to 12.6 years old). The detailed animal data are listed in the Appendix (Table 33). The effect of the storage duration until further processing and the ambient temperature on whole blood samples is summarized in Tables 21 and 22. The results of the measured analytes in samples stored at 22°C and 4°C were compared to the initial measurements and the data were analyzed by Kruskal-Wallis test, the significance was set at $p<0.05$.

The pH-values of the samples kept at room temperature (22°C) showed a significant decrease at 24 hours following sampling (7.36±0.02) and onward compared to initial measurements (7.38±0.01). The pH-values did not show a statistical difference at 4°C at either time point of determination (up to 72 hours) (Figure 19). The Base Excess showed a significant reduction in samples kept at room temperature (22°C) at 24 hours following sampling (0.6±1.47 mmol/l) compared to the results of the initial measurements (3.14±0.56 mmol/l). The results of Base Excess obtained for samples kept at 4°C did not show significant differences when compared to initial measurements at either time point of determination (up to 72 hours) (Figure 22). The pCO_2 in samples stored at both degrees of temperature (22°C and 4°C) showed no significant differences at either time point of determination (up to 72 hours) compared to the initial measurements. Although there was an obvious increase in pCO_2 for samples kept at 22°C which does not evidence statistical difference (Figure 20). Bicarbonate concentrations decreased significantly for samples stored at 22°C at 48 hours (24.9±1.67 mmol/l) following sampling compared to the initial measurements (28.08±0.64 mmol/l). Moreover, statistical differences were not observed in bicarbonate concentration for samples kept at 4°C compared to initial measurements (Figure 21).

A significant reduction in the concentration of glucose was observed for samples kept at room temperature (22°C) at 24 hours following sampling (1.68±0.32 mmol/l) compared to the results of the initial measurements (3.98±0.27 mmol/l). Moreover, results obtained for samples kept at 4°C

did not show significant differences at either time point of determination (up to 72 hours) when compared to initial measurements (Figure 23).

Blood lactate concentrations of the samples kept at both temperatures tend to increase significantly with time (Figure 24). Compared to initial measurements (0.674±0.11 mmol/l), blood lactate was found to increase significantly at 24 hours (3.92±0.42 mmol/l) and 48 hours (1.55±0.2 mmol/l) for the samples stored at 22^{o}C and 4^{o}C respectively.

Plasma potassium ion concentrations were significantly increased at room temperature (22^{o}C) at 24 hours following sampling (4.88±0.11 mmol/l) compared to the initial measurements (3.84±0.11 mmol/l), while results in samples kept at 4^{o}C showed a significant increase at 72 hours (5.82±0.23 mmol/l) compared to results of the initial measurements (Figure 25). Sodium ion concentrations in samples kept at 22^{o}C decreased significantly at 48 hours (139.6±0.42 mmol/l) compared to the initial measurements (141.68±0.49 mmol/l). Moreover, sodium ion concentrations of samples kept at 4^{o}C showed no significant differences at either time point of determination (up to 72 hours) (Figure 26).

The changes in sodium and potassium concentrations by the effect of storage time and temperature might be associated with the changes in other blood analytes. Spearman correlation was used to find out the extent to which the changes in pH, pCO_2, HCO_3^-, Base Excess, glucose and lactate are associated with the changes in sodium and potassium concentrations, in cattle blood samples stored at 22^{o}C and 4^{o}C with time interval up to 72 hours. The results are summarized in Table 23.

Alterations of potassium and sodium concentrations in samples stored at two different temperatures (22^{o}C and 4^{o}C) are time dependent. A strong negative correlation was observed between potassium and sodium ion concentrations at room temperature (22^{o}C) (r_s = -0.8), whereas no observed correlation in samples kept at 4^{o}C.

By studying the relationship between pH-values and potassium and sodium concentrations in samples kept at 22^{o}C, we found a moderate negative correlation with potassium (r_s = -0.46) and a strong positive correlation (r_s = 0.66) for sodium. Whilst, at 4^{o}C a very weak negative correlation

(r_s = -0.18) and a moderate positive correlation (r_s = 0.44) were observed with potassium and sodium, respectively.

Moreover, the relationship between pCO_2 and potassium and sodium concentrations in samples kept at 22°C showed a weak positive correlation with potassium (r_s = 0.31) and a moderate negative correlation for sodium (r_s = -0.51). At 4°C there was a weak positive correlation with potassium (r_s = 0.33) and a weak negative correlation with sodium (r_s = -0.38).

The relationship between HCO_3^- and potassium and sodium concentrations in samples stored at 22°C were a strong negative correlation with potassium (r_s = -0.79) and a strong positive correlation with sodium (r_s = 0.74). Whereas, at 4°C a moderate positive correlation with potassium (r_s = 0.44) and very weak positive correlation with sodium (r_s = 0.16) was observed.

In addition, Base Excess showed a moderate negative correlation with potassium (r_s = -0.55) in samples stored at 22°C, and a strong positive correlation with sodium (r_s = 0.69) at the same temperature. At 4°C moderate positive correlation was observed with sodium (r_s = 0.46) and no correlation was observed with potassium (r_s = 0.01).

The relationship between glucose and potassium and sodium concentrations in samples kept at 22°C are summarized as strong negative correlation with potassium (r_s = -0.76) and a strong positive correlation with sodium (r_s = 0.9). At 4°C, a moderate negative correlation with potassium (r_s = -0.55) and no correlation for sodium (r_s = 0.04).

The relationship between blood lactate and potassium and sodium concentrations at 22°C, are summarized as strong positive correlation with potassium (r_s = 0.78) and strong negative correlation with sodium (r_s = -0.9). But, at 4°C a moderate positive correlation was found with potassium (r_s = 0.47), while no correlation for sodium.

Table 21. The effect of storage duration for various intervals on the values of pH, pCO_2, HCO_3^- and Base Excess measured by ABL5™ in ten heparinized whole blood samples from cattle. The storage temperature was 4°C and room temperature (22°C), respectively. The results in the table are expressed as mean values ± SE. The grey boxes indicate significant changes and the asterisk * = significance at $p<0.05$, ** = significance at $p<0.01$ and *** = significance at $p<0.0001$

Parameters	pH		pCO_2 (mmHg)		HCO_3^- (mmol/l)		Base Excess (mmol/l)	
Temp.	**22°C**	**4°C**	**22°C**	**4°C**	**22°C**	**4°C**	**22°C**	**4°C**
0 min	7.38±0.01		47.17±2.16		28.08±0.64		3.14±0.56	
20 min	7.4±0.01	7.4±0.01	46.8±1.78	47±1.6	28.2±0.95	28.1±0.97	3.7±0.88	3.6±0.94
35 min	7.39±0.01	7.39±0.01	47.2±1.5	47.1±1.44	27.9±0.98	27.9±0.88	3.1±0.88	3.3±0.86
50 min	7.39±0.01	7.39±0.01	48.2±1.61	47.7±1.59	28.3±0.91	28.3±0.9	3.2±0.9	3.6±0.94
65 min	7.41±0.02	7.41±0.01	44.9±2.84	46.1±1.46	27.4±1	27.9±0.97	3.3±0.8	3.7±0.88
80 min	7.39±0.01	7.39±0.01	45.2±2.34	46.1±1.66	26.7±0.66	26.8±0.53	2.4±0.37	2.5±0.45
95 min	7.38±0.01	7.38±0.01	46.3±2.14	47.8±1.93	26.7±0.55	27.4±0.61	2.3±0.44	2.7±0.44
24 hours	7.36±0.02*	7.4±0.02	48±3.81	47.7±2.48	25.8±1.69	28.6±1.73	0.6±1.47*	3.9±1.65
48 hours	7.3±0.01**	7.37±0.01	52.4±3.08	50.4±2.31	24.9±1.67*	28.5±1.86	-1.5±1.47**	3.2±1.67
72 hours	7.28±0.02***	7.35±0.02	55.8±3.81	53±2.05	24.5±0.65**	28±0.77	-2.3±0.73***	2.1±0.88

Table 22. The effect of storage duration for various intervals on potassium and sodium ion concentrations, glucose and blood lactate concentrations measured by ISE (EML™ 105) in ten heparinized whole blood samples from cattle. The storage temperature of the specimens was 4 and 22°C, respectively. The results in the table are expressed as mean values ± SE. The grey boxes indicate significant changes and the asterisk * = significance at $p<0.05$, ** = significance at $p<0.01$ and *** = significance at $p<0.0001$

Parameters	Potassium concentration (mmol/l)		Sodium concentration (mmol/l)		Blood glucose (mmol/l)		Blood lactate (mmol/l)	
Temp.	**22°C**	**4°C**	**22°C**	**4°C**	**22°C**	**4°C**	**22°C**	**4°C**
0 min	3.84±0.11		141.68±0.49		3.98±0.27		0.674±0.11	
20 min	3.91±0.11	3.89±0.11	141.6±0.47	141.6±0.52	3.98±0.26	4.02±0.26	0.79±0.13	0.73±0.12
35 min	3.84±0.12	3.81±0.12	141.7±0.49	141.7±0.49	3.96±0.28	4.03±0.26	0.83±0.13	0.76±0.13
50 min	3.84±0.11	3.79±0.12	141.5±0.5	141.6±0.49	3.92±0.28	3.94±0.28	0.94±0.14	0.77±0.12
65 min	3.95±0.11	3.88±0.11	141.5±0.5	141.8±0.55	3.85±0.28	3.95±0.25	0.95±0.14	0.77±0.13
80 min	3.87±0.13	3.81±0.12	141.6±0.49	141.8±0.51	3.82±0.27	4.02±0.27	1.02±0.16	0.82±0.13
95 min	3.86±0.11	3.8±0.12	141.4±0.47	141.6±0.49	3.73±0.22	3.97±0.24	1.11±0.18	0.83±0.13
24 hours	4.88±0.11*	4.23±0.11	140.5±0.52	141.9±0.5	1.68±0.32*	3.82±0.3	3.92±0.42**	1.06±0.2
48 hours	5.58±0.16**	4.69±0.2	139.6±0.42*	141.8±0.51	0.3±0.12**	3.77±0.27	6.05±0.5***	1.55±0.2*
72 hours	6.16±0.2***	5.82±0.23***	139.1±0.43**	140.7±0.55	0±0.02***	3.57±0.21	6.88±0.54***	1.87±0.28**

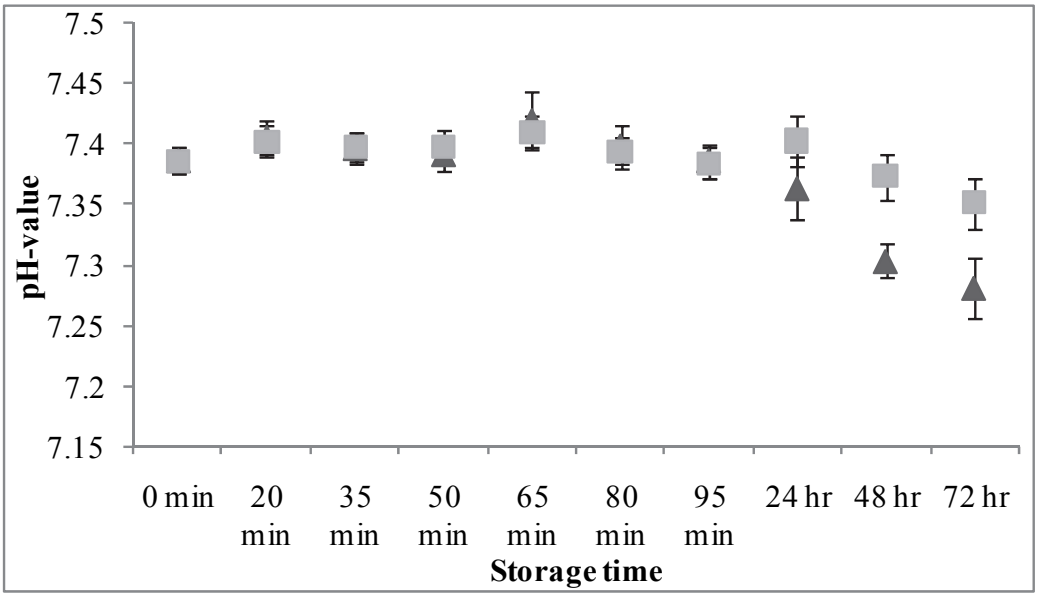

Figure 19. Effects of storage time and two different storage temperatures (4°C and 22°C) on the pH-values in ten heparinized whole blood samples from cattle as determined by Blood Gas Analyzer (ABL5™). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

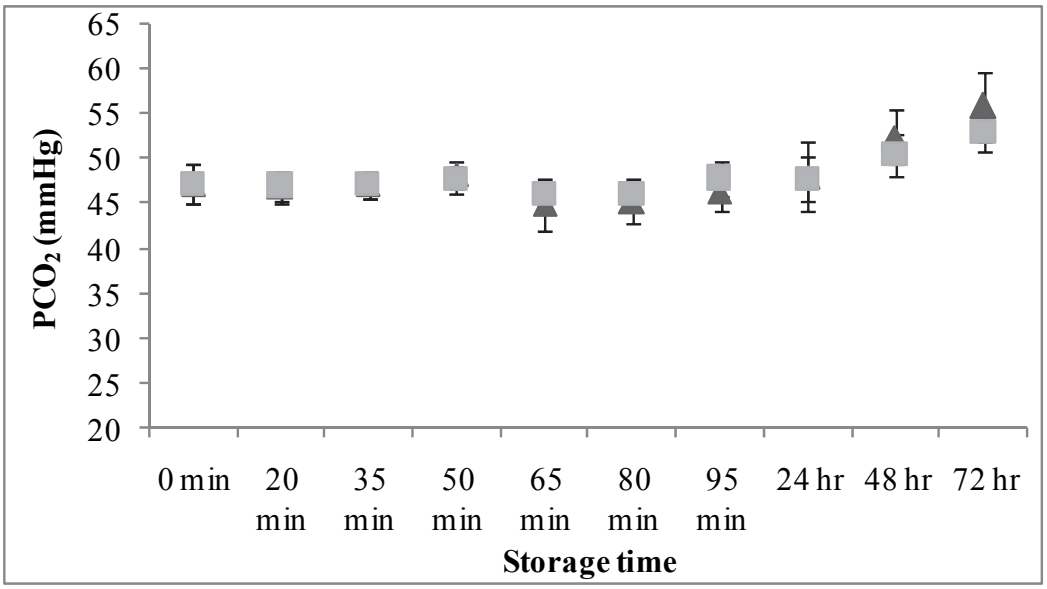

Figure 20. Effects of storage time and two different storage temperatures (4°C and 22°C) on the pCO_2 (mmHg) in ten heparinized whole blood samples from cattle as determined by Blood Gas Analyzer (ABL5™). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

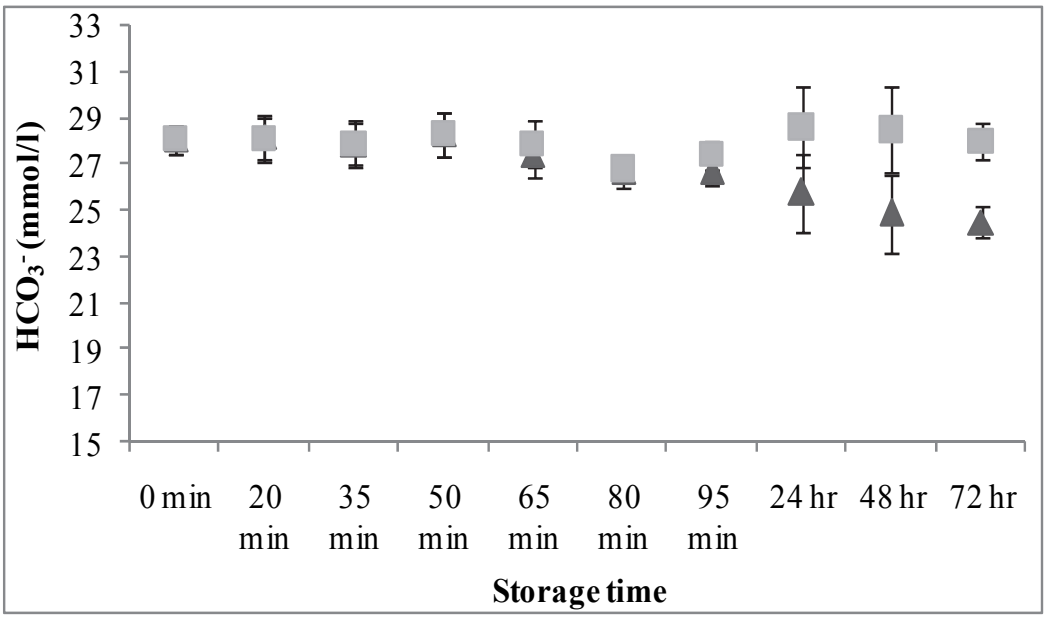

Figure 21. Effects of storage time and two different storage temperatures (4°C and 22°C) on the concentration of HCO_3^- (mmol/l) in ten heparinized whole blood samples from cattle as determined by Blood Gas Analyzer (ABL5™). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

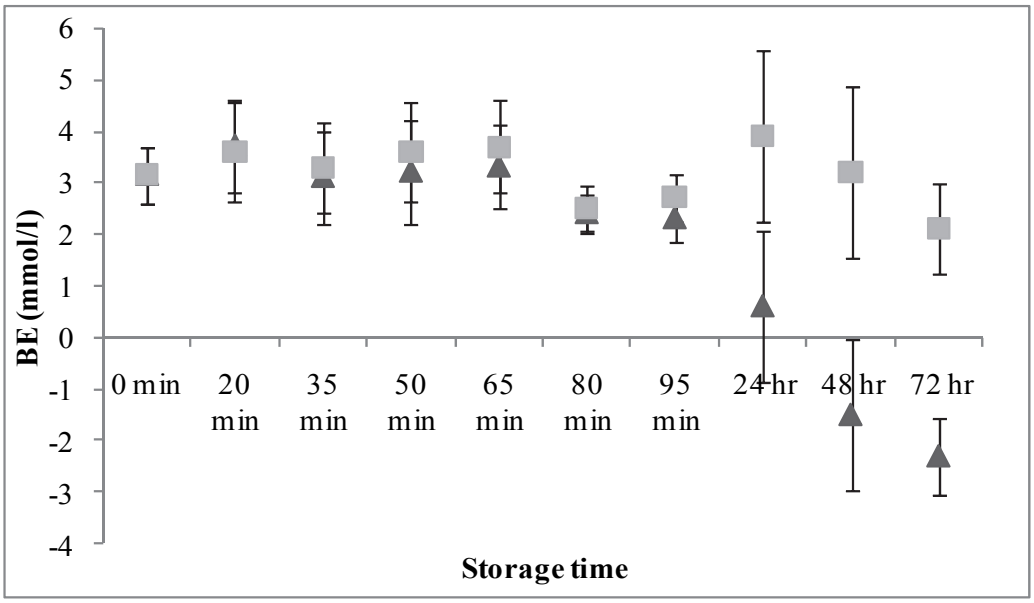

Figure 22. Effects of storage time and two different storage temperatures (4°C and 22°C) on the Base Excess (mmol/l) in ten heparinized whole blood samples from cattle as determined by Blood Gas Analyzer (ABL5™). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

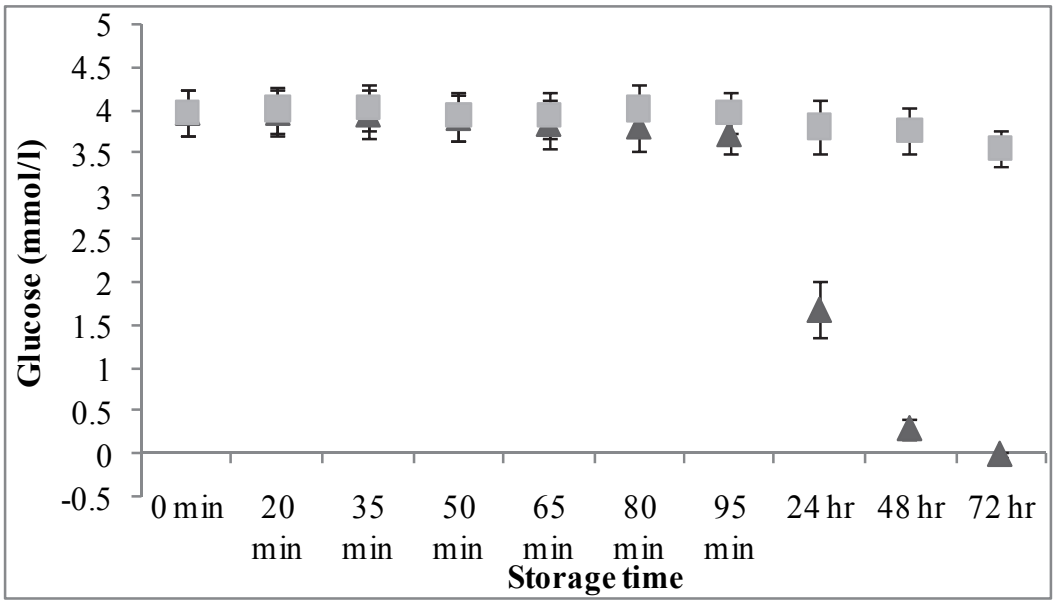

Figure 23. Effects of storage time and two different storage temperatures (4°C and 22°C) on glucose concentrations (mmol/l) in ten heparinized whole blood samples from cattle as determined by ISE (EML™ 105). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

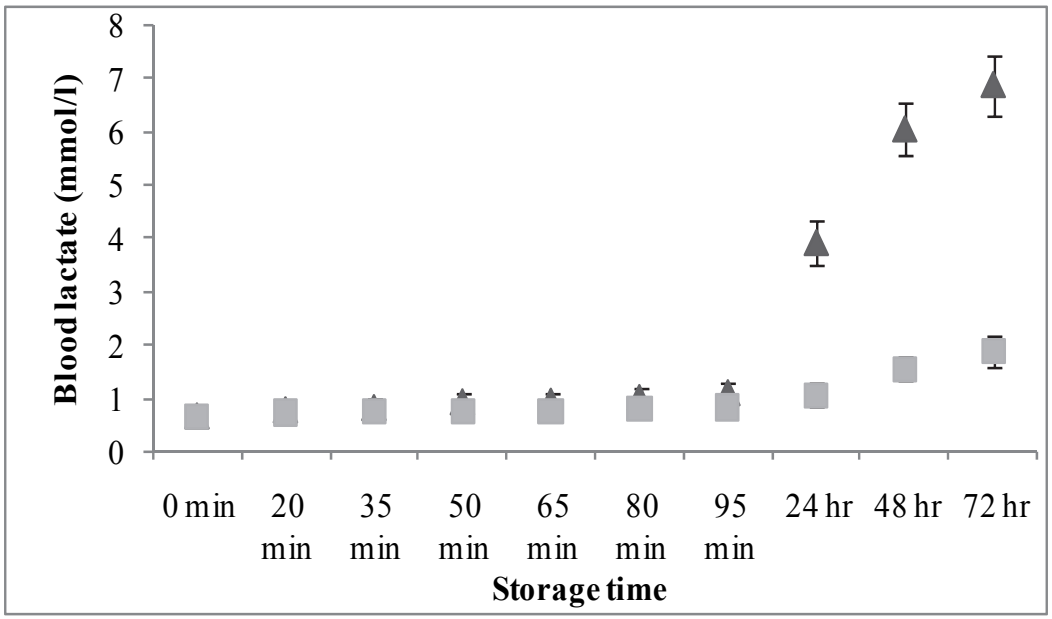

Figure 24. Effects of storage time and two different storage temperatures (4°C and 22°C) on blood lactate concentrations (mmol/l) in ten heparinized whole blood samples from cattle as determined by ISE (EML™ 105). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

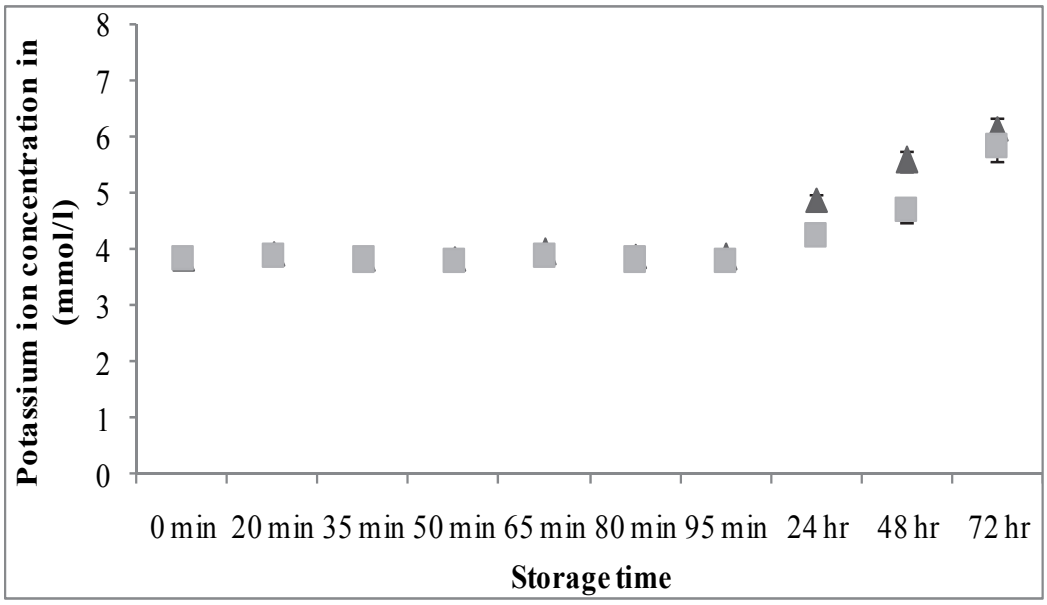

Figure 25. Effects of storage time and two different storage temperatures (4°C and 22°C) on potassium ion concentration (mmol/l) in ten heparinized whole blood samples from cattle as determined by ISE (EML™ 105). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

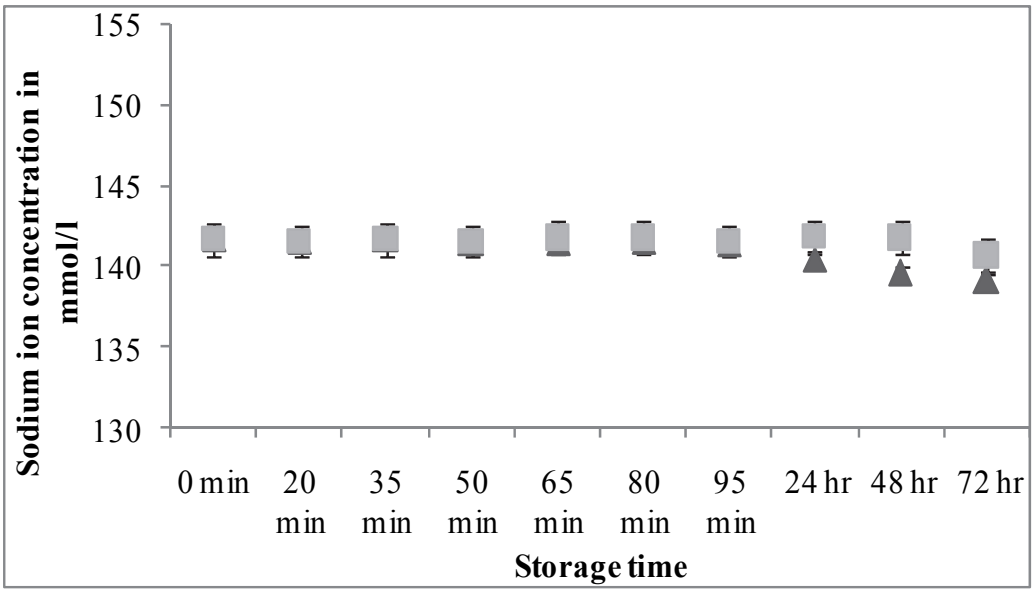

Figure 26. Effects of storage time and two different storage temperatures (4°C and 22°C) on sodium ion concentrations (mmol/l) in ten heparinized whole blood samples from cattle as determined by ISE (EML™ 105). Results are shown as mean ± SE. The ■ is at 4°C and ▲ is at 22°C

Table 23. Overview of the relationship between potassium and sodium ion concentrations and the values of pH, pCO_2, HCO_3^-, Base Excess, glucose and lactate concentrations in ten heparinized whole blood samples from cattle stored at room temperature (22°C) and at 4°C. The grey boxes indicate significant changes and the asterisk * = significance at $p<0.05$ and ** = significance at $p<0.01$. r_s = Spearman correlation coefficient

	Potassium concentration at 22°C [rs (p value)]	**Sodium concentration at 22°C [rs (p value)]**		**Potassium concentration at 4°C [rs (p value)]**	**Sodium concentration at 4°C [rs (p value)]**
pH at 22°C	-0.46 (0.187)	0.66 (0.037)*	**pH at 4°C**	-0.18 (0.62)	0.44 (0.2)
pCO_2 at 22°C	0.31 (0.379)	-0.51 (0.13)	**pCO_2 at 4°C**	0.33 (0.347)	-0.38 (0.286)
HCO_3^- (mmol/l) at 22°C	-0.79 (0.007)**	0.74 (0.014)*	**HCO_3^- (mmol/l) at 4°C**	0.44 (0.201)	0.16 (0.667)
Base Excess (mmol/l) at 22°C	-0.55 (0.102)	0.69 (0.028)*	**Base Excess (mmol/l) at 4°C**	0.01 (0.987)	0.46 (0.186)
Glucose (mmol/l) at 22°C	-0.76 (0.011)*	0.9 (0.000)**	**Glucose (mmol/l) at 4°C**	-0.55 (0.098)	0.04 (0.918)
Lactate (mmol/l) at 22°C	0.78 (0.008)**	-0.9 (0.000)**	**Lactate (mmol/l) at 4°C**	0.47 (0.171)	0.09 (0.811)
Potassium (mmol/l) at 22°C	-	-0.8 (0.006)**	**Potassium (mmol/l) at 4°C**	-	0.14 (0.693)
Sodium (mmol/l) at 22°C	-0.8 (0.006)**	-	**Sodium (mmol/l) at 4°C**	0.14 (0.693)	-

5.3 Trial III, effect of *in vitro* hemolysis on potassium ion concentration

Blood samples from twenty cattle, ten of the German Holstein breeds and ten cattle from the breed Belted Galloway (7 females and 3 males) with a mean age of 3.5 years (0.6 to 8.8 years old) were included in this study. On beforehand, the values of pH, pCO_2, HCO_3^-, Base Excess, potassium and sodium were shown to be within the reference range by laboratory analysis that preceded the present study. Animal data and laboratory results are given in detail in the Appendix (Table 34).

The dependent variable (y-axis) was defined as the difference in plasma potassium ion concentration (Delta K^+) between the hemolyzed (groups II and IV) and non-hemolyzed specimens (groups I and III) and the x-axis represents the plasma hemoglobin content. The obtained slope between the variables was the potassium correction factor within 95% confidence interval.

Hemolysis in bovine blood specimens affects the plasma potassium concentration. The plasma hemoglobin content applied to determine the grade of hemolysis ranged from 4.26 to 202.06 mmol/l (82.77±68.1 mmol/l) as shown in Table 24. In non-hemolyzed versus hemolyzed samples ranges of the potassium concentration were 3.7 to 7.5 mmol/l (4.99±1.14 mmol/l) and 4.0 to 8.3 mmol/l (5.24±1.18 mmol/l), respectively. The differences observed for potassium concentrations (Delta K^+) between hemolyzed (groups II and IV) and non-hemolyzed specimens (groups I and III) were 0.00 to 0.8 mmol/l (0.24±0.21 mmol/l). The higher value of free plasma hemoglobin was observed in hemolyzed samples evoked by multiple times of ejections with significant differences compared to those evoked by single ejection. The free plasma hemoglobin content in hemolyzed samples induced by single ejection was 4.26 to 119.63 mmol/l (38.10±35.33 mmol/l) while in hemolyzed samples induced by repeated ejections was 31.42 to 202.06 mmol/l (126.80±58.22 mmol/l).

The data of the dependent variable (y-axis) representing the difference in potassium concentration (Delta K^+) between the hemolyzed (groups II and IV) and non-hemolyzed specimens (groups I and III) are plotted against the plasma hemoglobin content (x-axis). A high

significant positive linear relationship ($p<0.0001$) existed between the change in potassium concentration and FPHgb from the non-hemolyzed to hemolyzed specimens (Figure 27). As apparent from the figure, the increase in plasma potassium concentration in hemolyzed specimens shows a linear relationship to plasma hemoglobin. The obtained slope between the variables was the potassium correction factor within 95% confidence interval. A correction factor for potassium concentration of 0.0025(at 95% confidence interval, 0.0018 to 0.0031) x FPHgb was obtained from simple linear regression using a best-fit line through zero.

Table 24. Potassium ion concentrations as determined by ISE (EML^{TM} 105) in 20 bovine plasma samples in specimens without and with *in vitro* hemolysis. The results are expressed as minimum, maximum and mean ± SD

	Min.	Max.	Mean ± SD
Potassium concentrations (mmol/l) in non-hemolyzed specimens (I, III)	3.70	7.50	4.99±1.14
Potassium concentrations (mmol/l) in hemolyzed specimens (II, IV)	4.00	8.30	5.24±1.18
Delta potassium (mmol/l) (Hemolyzed minus non-hemolyzed samples)	0.00	0.8	0.24±0.21
FPHgb (mg/dl) in hemolyzed specimens (II, IV)	4.26	202.06	82.77±68.1
FPHgb (mg/dl) in hemolyzed specimens (II, IV), single ejection	4.26	119.63	38.10±35.33
FPHgb (mg/dl) in hemolyzed specimens (II, IV), multiple ejection	31.42	202.06	126.80±58.22

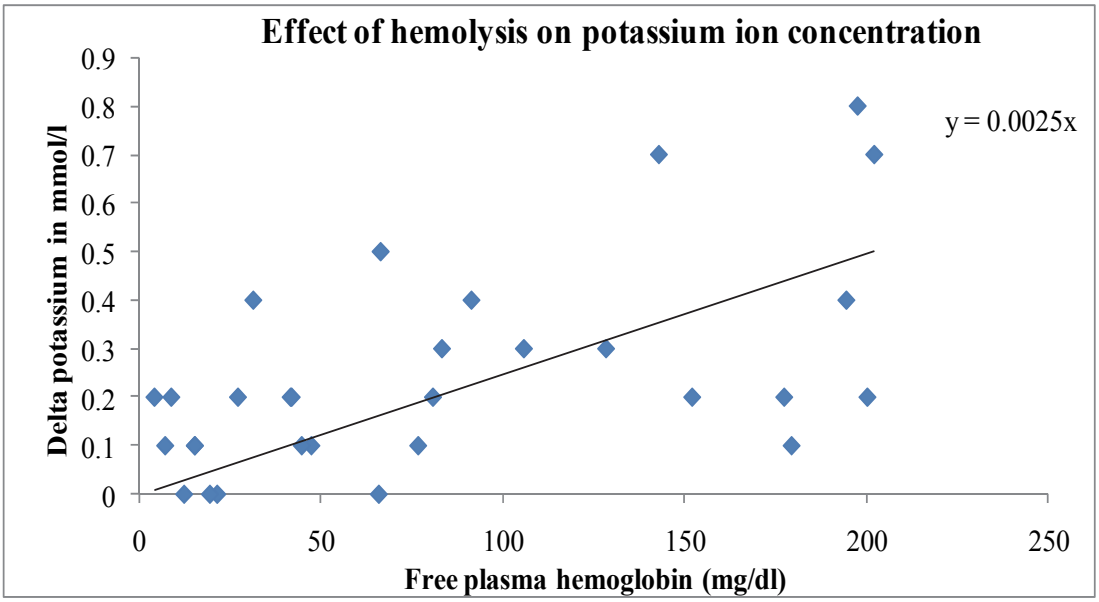

Figure 27. Effect of *in vitro* hemolysis on plasma potassium concentrations as determined by direct ISE. The delta potassium mmol/l (hemolyzed minus non-hemolyzed samples) is plotted against the free plasma hemoglobin as a measure for grade of hemolysis. The solid line showed a high significant positive linear relationship between them with a correction factor (0.0025 x FPHgb) and at 95% confidence interval was 0.0018 to 0.0031

5.4 Trial IV, the relationship between muscle and plasma potassium concentrations

A total of 13 plasma and muscle samples obtained from 13 German Holstein cattle admitted to the Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin were used in this study. The ages ranged from 1 month to 2 years. The cattle suffered from various disorders and were euthanized due to fatal prognosis following the decision of the owner. The detailed data are listed in the Appendix (Table 35).

By investigating the relationship between muscle tissue potassium and plasma potassium concentrations, blood and muscle samples were collected immediately after euthanasia of the animals. Potassium concentration in both plasma and muscle biopsy samples were 4.01±0.72 mmol/l and 89.59±11.87 mmol/kg wet weight, respectively and the concentration of sodium in both plasma and muscle biopsy samples were 136.23±3.22 mmol/l and 86.78±38.66 mmol/kg wet weight, respectively. The values of pH, pCO_2, HCO_3^- and Base Excess were 7.38±0.07, 48.92±6.38 mmHg, 27.92±3.33 mmol/l and 2.92±4.11 mmol/l, respectively (Table 25).

Plotting the results of sodium and potassium concentration in muscle tissue biopsy against the measurements of the plasma sodium and potassium concentration of the same samples showed very weak correlation between the potassium muscle content and the plasma potassium concentration ($r = 0.06$). Similarly, there was a very weak correlation observed between the muscle sodium content and plasma sodium concentration ($r = -0.09$) (Figures 28, 29 and Table 26).

By investigating the effect of plasma pH-value on the concentration of potassium and sodium in both plasma and muscle biopsy samples, we found weak correlation between plasma pH-values and plasma potassium concentration ($r = 0.32$). While, no correlation observed between plasma pH-values and muscle potassium content ($r = 0.01$) (Figure 30). There was weak correlation between plasma pH-values and plasma sodium concentration ($r = 0.3$) and between plasma pH-values and muscle sodium content ($r = 0.21$) (Figure 31).

Table 25. Mean ± SD, minimum and maximum values of sodium and potassium ions concentration measured by ISE (EML™ 105) in plasma and by Atomic absorption spectroscopy (contrAA 700) in muscle biopsy samples and blood gases measured by Blood Gas Analyzer (ABL5™) in 13 whole blood samples of cattle under investigation, the results are expressed as mean values ± SD

	n=	Minimum	Maximum	Mean ± SD
pH-values	13	7.20	7.48	7.38±0.07
pCO_2 (mmHg)	13	39.00	61.00	48.92±6.38
HCO_3^- (mmol/l)	13	18.00	31.00	27.92±3.33
Base Excess (mmol/l)	13	-10.00	6.00	2.92±4.11
Plasma potassium concentration (mmol/l)	13	2.50	4.90	4.01±0.72
Plasma sodium concentration (mmol/l)	13	129.00	141.00	136.23±3.22
Muscle potassium concentration (mmol/kg)	13	72.03	108.93	89.59±11.87
Muscle sodium concentration (mmol/kg)	13	43.10	184.67	86.78±38.66

Table 26. The relationship between the blood gas analysis parameters and the concentration of potassium and sodium in plasma and muscle biopsy samples from 13 cattle, the results were expressed as the Pearson correlation coefficient and the significance, r (p value). r = Pearson correlation coefficient. The asterisk * = significance at $p<0.05$

	Plasma potassium concentration (mmol/l)	**Plasma sodium concentration (mmol/l)**	**Muscle potassium concentration (mmol/kg)**	**Muscles sodium concentration (mmol/kg)**
pH-values	0.32 (0.287)	0.3 (0.312)	0.01 (0.967)	0.21 (0.481)
pCO_2 (mmHg)	0.39 (0.191)	-0.1 (0.754)	0.34 (0.258)	-0.32 (0.291)
HCO_3^- (mmol/l)	0.63* (0.021)	0.27 (0.379)	0.32 (0.279)	0.01 (0.983)
Base Excess (mmol/l)	0.6* (0.032)	0.27 (0.368)	0.29 (0.338)	0.03 (0.914)
Muscle potassium concentration (mmol/kg)	0.06 (0.850)	-	-	-
Muscles sodium concentration (mmol/kg)	-	-0.09 (0.763)	-	-

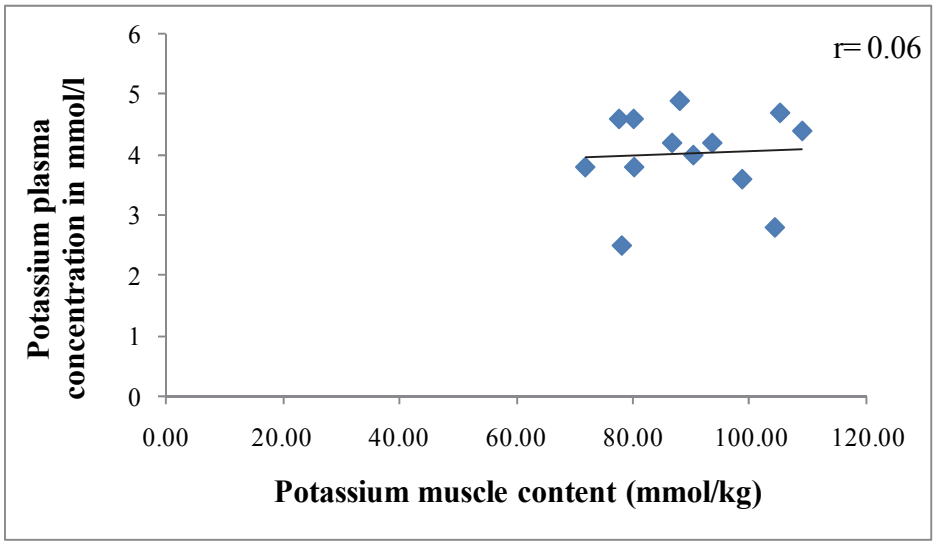

Figure 28. The correlation between potassium concentration in muscle and plasma samples of 13 cattle. The graph illustrates a very weak correlation (r = 0.06) between muscle potassium content in mmol/kg based on muscle wet weight and the plasma potassium concentration in mmol/l. r = Pearson correlation coefficient

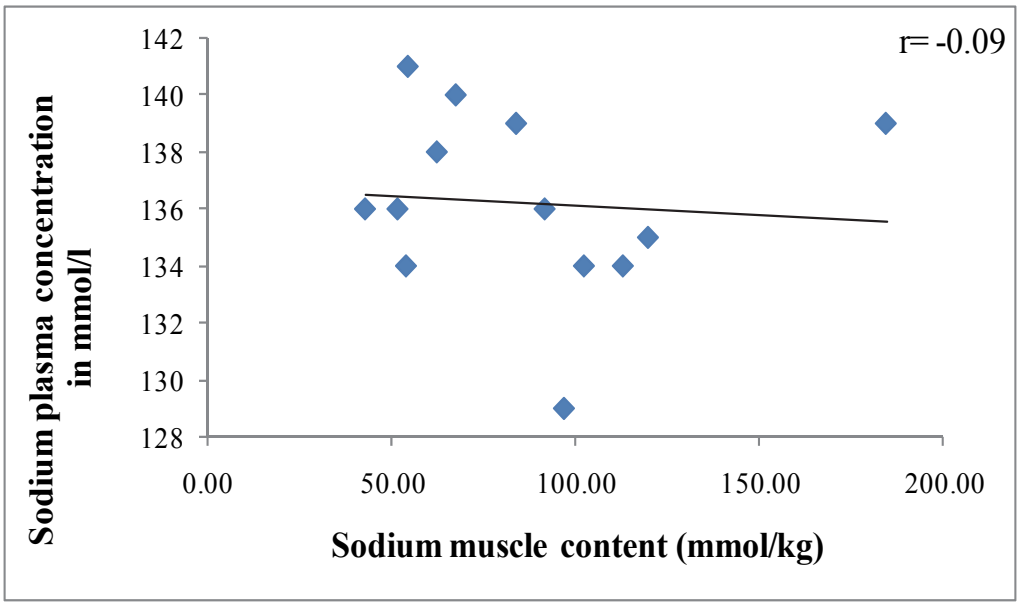

Figure 29. The correlation between sodium concentration in muscle and plasma samples of 13 cattle. The graph illustrates a very weak negative correlation (r = -0.09) between muscle sodium content in mmol/kg based on muscle wet weight and the plasma sodium concentration in mmol/l. r = Pearson correlation coefficient

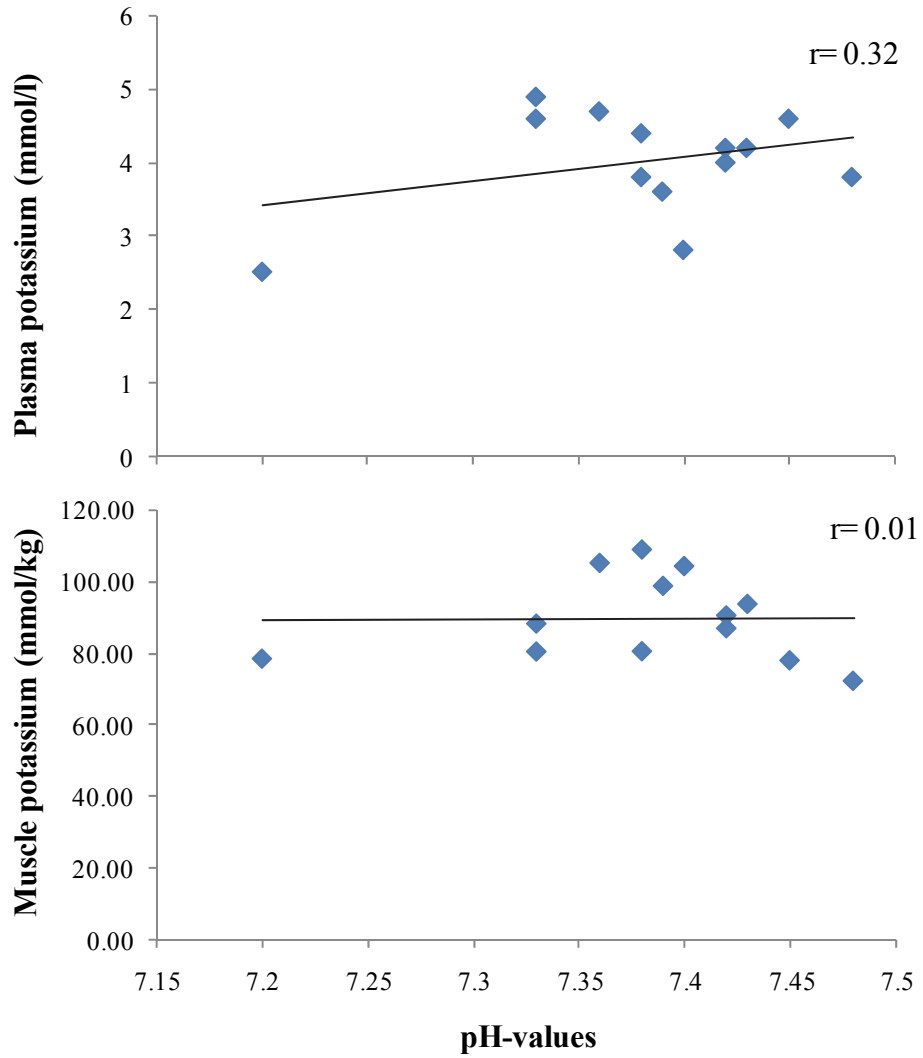

Figure 30. The relationship between plasma pH-values and the concentration of potassium in plasma (mmol/l) and muscle biopsy samples (mmol/kg wet weight) obtained from 13 cattle used to investigate the relationship between plasma and muscle potassium concentration. The graph illustrates a weak correlation between plasma pH-values and plasma potassium concentration (r = 0.32) and no observed correlation between plasma pH-values and muscle potassium content (r = 0.01). r = Pearson correlation coefficient

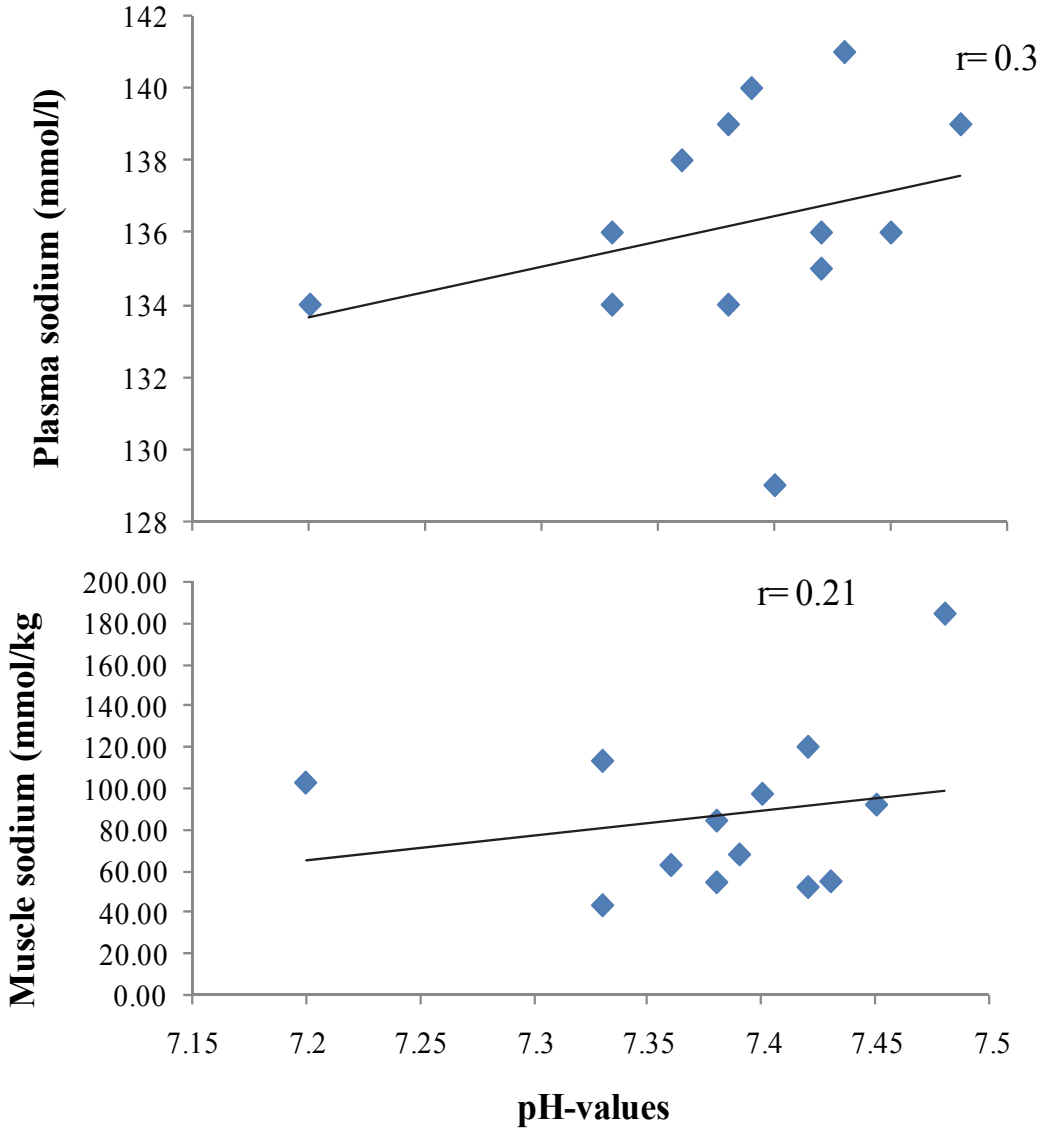

Figure 31. The relationship between plasma pH-values and the concentration of sodium in plasma (mmol/l) and muscle biopsy samples (mmol/kg wet weight) obtained from 13 cattle used to investigate the relationship between plasma and muscle sodium concentration. The graph illustrates a weak correlation between plasma pH-values and plasma sodium concentration (r = 0.3) and between plasma pH-values and muscle sodium content (r = 0.21). r = Pearson correlation coefficient

6 Discussion

Until today flame photometry has been the golden standard for measurement of electrolyte concentrations in various substrates among these electrolytes sodium and potassium. In recent years, analysis by direct ISE has become more and more popular, as this technology allows immediate determination of potassium and sodium ion concentrations in undiluted blood samples. As the analytical procedure is easy, staff members of a clinic or even practitioners in the field are able to operate the machine on their own. Flame photometry, indirect and direct ISE, however, have been demonstrated to underlie several interferences. Interference occurs when a substance or process leads to a false result in measurement technique which subsequently could lead to misinterpretation of the results by the veterinarian (Dimeski et al., 2010). Numerous studies have been performed that deal with various causes for interferences in the course of ISE measurements in humans such as the pre-analytical processing, therapeutic compounds, sample matrix and processing during measurement etc. (Dimeski et al., 2010). Although ISE are widely used in veterinary medicine, in particular in small animal and equine practice as well as in experimental settings, only scarce reports can be found dealing with the use of ISE in veterinary medicine.

6.1 Trial I, agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™ 105)

According to the convention of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the results of ISE measurements of sodium and potassium in undiluted plasma should be reported in terms of substance concentration (mmol/l) in order to be comparable with the results obtained by application of the Golden Standard flame photometry (Burnett et al., 2000). Maas et al. (1985) reported that the activities measured by ISE for sodium and potassium ion concentrations in human blood samples should be multiplied by an appropriate factor depending on the water mass concentration of the sample to reflect the same values (total substance concentration) obtained by the flame photometry as a substance concentration obtained by ISE in normal plasma samples. The fact that, flame photometer and direct ISE measure sodium and potassium in diluted and undiluted blood samples, respectively (Apple et al., 1982), seems to justify the request of a correction factor. The ISE (EML™ 105; Radiometer Copenhagen, Copenhagen, Denmark) is a direct ISE instrument with an integrated conversion algorithm to

report results in concentration terms that are comparable to those obtained by flame photometry. This is true, however, only for specimens with normal plasma water (CLSI, 2000). FAES are standardized assuming a normal concentration of 7% solids (total protein and lipids) and 93% water content in plasma samples. To this end, results on standard normal samples examined by ISE should deliver identical values as FAES on diluted samples. Standard normal plasma specimens are defined as having a mass concentration of water, plasma total CO_2, plasma pH and concentrations of total proteins and cholesterol within the given range (Burnett et al., 2000).

A series of 15 replicates using two control sera [level 2 "sodium 138 mmol/l and potassium 4.41 mmol/l" and level 3 "sodium 153 mmol/l and potassium 6.32 mmol/l"] analyzed by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) demonstrated a within-run imprecision (CV%) between 0 and 0.93% for sodium and potassium (FAES and ISE) and the mean differences (Bias) of the ISE (EML™ 105) to FAES (AAS, SOLAAR M6) for the level 2 and level 3 quality control were 0.06 mmol/l and -0.15 mmol/l for potassium and -0.57 mmol/l and -0.25 mmol/l for sodium. Our results were in agreement with those recommended by the requirements set by IFCC and CLSI where the coefficient of variation (CV%, within-run imprecision) should be less than or equal 1.5% at both level of the control sera on both analyzers and the deviation of ISE from FAES should be no larger than ±1 mmol/l to achieve a bias ±2% at 95% confidence interval (Burnett et al., 2000, CLSI, 2000).

Bland and Altman agreement analysis were used for the data obtained (Bland and Altman, 1999). The 95% limit of agreement, estimated by mean difference ± 1.96 standard deviation of the differences, provide an interval of 95% of differences between potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) are found to lie between the upper and lower limits of agreement whilst, 5% of the values were outside the limit. This means that, almost all pairs of measurements of each analyte by the two analyzers will be closer together than the extreme values which provide 95% limits of agreement that proves appreciable agreement in the measurement of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) in blood samples with normal plasma protein and lipid concentrations. Achieving a 95% limits of agreement between FAES (AAS, SOLAAR M6) and direct ISE (EML™ 105) for determination of sodium and potassium in bovine blood samples reflect the validity of using the constant factors applied on human blood and that were integrated automatically according to the

manufacturer's manual into the ISE (EML™ 105; Radiometer Copenhagen, Copenhagen, Denmark) for bovine blood samples.

Our results are in agreement with a previous study performed by Albert et al. (2011) and Sharma and Sarmah (2013) who carried out an agreement analysis between flame photometer (NFP 460) and direct ISE (Vitros 350) analyzers for measurements of sodium and potassium in human blood samples. The authors report a 95% degree of agreement between the results obtained by use of the two instruments when measuring sodium and potassium in samples with normal serum protein and lipid concentrations. They attributed the differences in results obtained by FAES and ISE to the fact that diluted (in FAES) and undiluted samples (in ISE) are used. The same observations were reported by Preusse and Fuchs (1979) who compared ion selective electrodes and flame photometry for determination of sodium and potassium in human serum for clinical purposes, and concluded that ISE (ORION SPACE-STAT,SS-30 and TECHNICON STAT/ION) can be used for the routine estimations of sodium and potassium in clinical laboratories.

In previous studies a fair degree of agreement between the flame photometry and direct ISE methodologies in blood samples from humans was achieved. The authors concluded that the FAES would be suitable in small scale hospitals, whilst, ISE would be suitable for use in medium and large scale hospitals, because of their precision, rapid, simple and automated method which is not affected by the presence of alterations in total protein and lipid concentrations (Owens et al., 2005, Mikolaenko et al., 2000, Worth, 1985, Pelleg and Levy, 1975).

Changing the concentrations of the solid part in plasma alters the water content of the sample due to water displacement. In addition, sodium and potassium in plasma with high values of protein content are attracted to the protein fraction due to their positive electric charge. This phenomenon results in unreliable results from FAES (Kulpmann, 1989) reflected by a false increase (pseudohyper-) or decrease (pseudohypo-) in sodium and potassium values in samples with low or high total protein content or -lipidemia. These disadvantages are eliminated by direct ISE technology (Dimeski et al., 2005, Lang, 2002, Apple et al., 1982).

Our findings concerning the effect of variable concentrations of plasma total proteins, albumin and globulins on the results of potassium and sodium measurements by FAES (AAS, SOLAAR

M6) and ISE (EML™ 105) showed that low total protein levels tended to cause a pseudohyper-effect in both potassium and sodium and the reverse, a pseudohypo- effect with high concentration occurred. The major effect was based mainly on alterations in globulin concentrations but not albumin concentrations where it showed no correlation with the differences in the results of potassium and sodium obtained by the two analyzers. These results were in agreement with those obtained by Dimeski and Barnett (2005) for total protein and globulin but not for albumin and this may be attributed to the level of albumin in the study samples which were not extremely high or low.

The effect of variable concentrations of plasma cholesterol on the results of measurements of potassium and sodium by FAES (AAS, SOLAAR M6) and ISE (EML™ 105) in the present study showed a non-linear indirect weak correlation between the cholesterol concentration and the differences in the results of potassium measurements while very weak correlation was found for sodium by the two methodologies. In cattle, lipid concentrations in peripheral blood are not as high as in other animal species (e.g. the horse) and man. A greater effect would have to be expected for measurements using FAES in plasma samples obtained from ponies suffering from hyperlipidemia while cattle mobilizes non-esterified fatty acids from fat tissues into the circulation and only comparably small amounts of lipoproteins and triglycerides (Lascelles, 1965).

We tested if plasma pH would have an impact on the results of potassium and sodium measurements by induction of alterations in charge on proteins which could cause differences in binding of potassium and sodium, but came to the conclusion that no such effect can be proven by our experiments. Our findings showed a very weak correlation between the pH values and the differences in the results of potassium measurements while weak correlation was found for sodium by the two methodologies. Our results are in accordance with observations by Dimeski and Barnett (2005) who found that the correlation coefficients for the pH vs. direct and indirect ISE differences (the latter requires a dilution step as for FAES) in sodium, potassium and chloride measurements were < 0.02, indicating that the pH had no significant very weak effect on the results, where significant changing in potassium and sodium measurements requires extreme changes in plasma pH-values as in severe metabolic acidosis, where potassium is redistributed from the intra- to the extracellular space in exchange with H^+ through the cell membrane to

maintain electro-neutrality (Radostits and Done, 2007, Rastegar and Soleimani, 2001, Adrogue and Madias, 1981). Also, our findings are in contrast to a report by Russell et al. (1988) who concluded that instruments applied to measure sodium and potassium concentrations in undiluted plasma agreed with the outcome of flame emission spectrometry only if samples have a normal plasma pH.

In conclusion, the results of Trial I confirmed that differences found between the results obtained by FAES (AAS, SOLAAR M6) and direct ISE (EML™ 105) for the same blood samples are clearly associated with the total protein and cholesterol concentrations in these samples. Most probably, these differences are related to the pre-analytical dilution step in FAES causing alterations in the volume of the non-aqueous phase and water displacement with increasing protein and lipid concentrations. The latter finding is not present when direct ISE is used as the latter technology relies on the determination of the ion activity in the water phase of whole blood which contains the dissolved electrolytes, which are not influenced by the presence of variable concentrations of total protein and lipids. A study by Lang (2002) using equal numbers of samples with low and high protein concentrations stated that the erroneous sodium results owing to the use of an indirect ISE was 2% in hypoproteinaemic and 20% in hyperproteinaemic samples. In a previous study including indirect ISE, substantial differences were existed between the results reported by direct and indirect ISE in one of four samples from human patients in intensive care units and in one of twelve samples from hospitalized patients (Dimeski et al., 2012). These authors identified overestimation associated with hypoproteinemia, potentially leading to false classification of the sodium content of the samples when indirect ISE was applied, whereas the same authors suggest using direct ISE to prevent such interferences due to alterations in water mass concentration in diluted samples. The same effect is reported for cases of severe hyperlipemia in humans (Maas et al., 1985).

6.2 Trial II, pre-analytical factors (effect of storage duration and temperature)

The quality of the analytical process in certified laboratories has improved substantially in recent years due to high standards applied to the quality control management; the pre-analytical process most often does not underlie any quality control and for this reason has greater impact on the laboratory result than the processing of the sample in the laboratory itself. Various precautions

with respect to handling of the blood sample, starting at its collection and ending at the laboratory, are required for obtaining reliable results. The shortest time between sample collection and its analysis in addition to storage of the blood samples on ice are purposing an ideal analysis (Brito et al., 2008, Gokce et al., 2004, Szenci et al., 1991, Szenci and Besser, 1990). Delay in plasma harvesting and variations in storage temperature provoke changes in blood gas composition and acid-base values as a result of continued temperature-depending anaerobic and aerobic metabolism in the period from sample collection until analysis (Beaulieu et al., 1999, Muller-Plathe and Heyduck, 1992, Szenci et al., 1991). At low temperatures (4ºC), the metabolic processes within the blood slow down and the energy consumptive activity of the Na^+/K^+-ATPase pump is decreased (Brito et al., 2008, Gokce et al., 2004).

In the present study, the pH-values of samples kept at room temperature (22^{o}C) showed a significant decrease at 24 hours from sampling and onwards, whereas no significant differences were observed for the blood pH-value, when samples were kept at 4^{o}C at either time point of determination (up to 72 hours). The significant reduction of the blood pH-values for samples kept at room temperature (22^{o}C) is due to continuation of metabolism in blood cells with glucose utilization and the formation of carbon dioxide and water or lactic acid, after oxygen supply has stopped and the metabolism has switched to anaerobic glycolysis. Maintaining the pH-values in samples stored at 4^{o}C has been attributed to deceleration of temperature dependent metabolic processes. In a previous study by Brito et al. (2008), no statistical significant differences were observed between the pH in rat blood samples stored at room temperature and those stored on ice for up to 95 minutes. Gokce et al. (2004) studied the effect of time delay to measurement and storage temperature on blood gas and acid-base values of bovine venous blood. In the latter studies, pH-values of samples kept at different temperatures (4, 22 and 37^{o}C) were significantly decreased with time (after 5, 3 and 2 hours). Similar results were achieved by Poulsen and Surynek (1977), who stated that, the pH-values of blood samples collected from cattle and stored at 4^{o}C remained stable for a period of 5-6 hours.

Our results did not show any significant differences in pCO_2 concentration in samples kept at room temperature (22^{o}C) and on ice (4^{o}C) at either time point of determination (up to 72 hours). Although, there was an obvious increase observed for pCO_2 in samples kept at room temperature the results did not reach statistical significance. The increase in pCO_2 can be attributed to an

increase in the generation of carbon dioxide via aerobic metabolism of glucose resulting in the production of carbon dioxide and water as long as sufficient oxygen is present (Liss and Payne, 1993). Our results are in agreement with previous observations by Brito et al. (2008), who found no statistical relevant difference when measuring pCO_2 in blood samples from rats stored on ice up to 95 minutes. Our observations, however, are in disagreement with those obtained by Gokce et al. (2004). The latter researchers found that the mean pCO_2 values in blood stored at 4^{o}C decreased significantly until 24 hours following sampling, but increased again to initial levels while, the pCO_2 values of the samples kept at 22 and 37^{o}C increased significantly after the fourth hour and continued to increase afterwards.

Our results showed significant decreases in HCO_3^- concentrations in samples kept at room temperature (22^{o}C) at 48 hours, while samples kept at 4^{o}C showed no significant difference at either time point of determination (up to 72 hours). The reduction in HCO_3^- in samples kept at room temperature was attributed to the formation of lactic acid with the generation of hydrogen ions from anaerobic glycolysis that are buffered by bicarbonate ions (Liss and Payne, 1993). These findings are in agreement with those obtained by Gokce et al. (2004) who found that, the bicarbonate concentrations decreased significantly in samples kept at 4^{o}C and 22^{o}C with the most prominent decrease at 37^{o}C. In previous study by Brito et al. (2008), the results showed no statistically relevant difference in HCO_3^- concentrations in rat blood samples stored on ice up to 95 minutes.

The results of Base Excess values revealed that, samples kept at room temperature (22^{o}C) showed a significant decrease at 24 hours following sampling. In contrast, at 4^{o}C the Base Excess did not show changes at either time point of determination (up to 72 hours). In a previous study by Gokce et al. (2004), the Base Excess concentrations were shown to decrease significantly in all bovine blood samples stored at 4^{o}C, 22^{o}C and 37^{o}C, the most prominent decrease in Base Excess concentrations was recorded for the samples kept at 37^{o}C compared to the baseline values. The explanation for this finding is that the buffering capacity in blood declines with lactic acid formation in blood samples stored at room temperature.

The glucose levels showed a significant reduction in samples kept at room temperature (22^{o}C) first observed at 24 hours and then at any of the subsequent samples, whereas the alterations of

glucose in samples kept at 4°C were not significant at either time point of determination (up to 72 hours). In parallel, the blood lactate levels significantly increased at 24 hours following sampling and onwards when samples were stored at room temperature (22°C), while, blood lactate in samples kept at 4°C significantly increased at 48 hours of the initial measurement. The changes in glucose and lactate concentrations with time and temperature are attributed to the presence of RBCs which resulted in continued uptake of glucose and its utilization via the aerobic pathway in first instance followed by anaerobic glycolysis after oxygen supply ceased. Thus based on our findings we can support the conclusions of other authors (Schulze, 2008, Christopher and O'Neill, 2000) that centrifugation of samples before posting should be preferred to storing the samples on ice. RBCs and white blood cells are removed by this step such that no interference with the metabolism of these cells occurs that could lead to misinterpretation of results of the laboratory.

The present study showed significant increases in potassium concentrations at both storage temperatures; when blood samples stored at room temperature (22°C) the increase in potassium levels was evident at 24 hours following initial measurements, while samples kept at 4°C showed a significant increase at 72 hours. On the other hand, sodium ion concentrations in samples kept at room temperature significantly decreased at 48 hours following sampling and - when stored at 4°C- did not show significant differences at either time point of determination (up to 72 hours) when compared to initial measurements. The increase in potassium ion concentrations in samples kept at 22°C was associated with decreasing sodium concentrations as reflected in a significant strong negative correlation between the two analytes.

The observed alterations of potassium and sodium at room temperature (22°C) are either attributable to the functions of the sodium-potassium pump, or to alterations in the acid-base homeostasis. The sodium-potassium pump is maintaining the potential difference between the extra- and the intracellular space by moving sodium and potassium ions across the cell membrane through an active transport process involving the hydrolysis of ATP to provide the necessary energy. This process is responsible for maintaining the large excess of sodium ions outside the cell and the large excess of potassium ions inside the cell. In samples stored at room temperature (22°C) the pump is activated due to increased production and utilization of ATPase, followed by a decreasing activity of the pump at a later stage when the glucose levels decrease. As a consequence, sodium enters the cells via diffusion and potassium leaves the cell by the

concentration gradient leading to changes in potassium and sodium levels in the plasma or serum phase of whole blood samples (Negendank and Shaller, 1982). As potassium is present in the extracellular space in much smaller concentrations than sodium, changes in potassium levels due to dysfunction of the sodium/potassium pump become more obvious.

At the same time, the continuing metabolic processes in the blood cause an increase in the generation of CO_2 via the aerobic metabolism. Within the tube, carbon dioxide reaches the gas phase that covers the blood sample in the tube, leading to a reduction in pCO_2, followed by its increase after the gas pressure has reached an amount that forces carbon dioxide to dissolve in blood again. After oxygen supply has ceased, lactate is formed through the process of anaerobic glycolysis which decreases the pH-value and HCO_3^- concentration (Aronson and Giebisch, 2011). The effect of reduction in pH-values (increasing concentrations of hydrogen ions) that cause potassium loss from the cells is often attributed to a membrane K^+-H^+ exchange. The predominant pH regulatory pathway "Na^+-H^+ exchange" leading sodium to enter the cell by this pathway forced out through the Na^+, K^+- ATPase. Consequently, the cellular uptake of potassium by the Na^+, K^+- ATPase will be greater when Na^+-H^+ exchange activity is stimulated and will be diminished when the rate of Na^+-H^+ exchange is reduced. Lowering the extracellular pH would result in inhibition of the rate of Na^+-H^+ exchange, leading to the accumulation of intracellular H^+ and a decline in intracellular sodium causing reduction in Na^+, K^+- ATPase activity, with decreasing the active cellular uptake of potassium (Aronson and Giebisch, 2011, Brito et al., 2008, Gokce et al., 2004).

At 4^{o}C storage temperature, minimal alterations in extracellular potassium and sodium ion concentrations were attributed to a diminished activity of the cellular metabolic processes at low temperatures in detail the functioning of energy dependent channel systems (Negendank and Shaller, 1982). Our findings are in disagreement with the results observed by Brito et al. (2008), who found statistically relevant differences for potassium and sodium in blood samples from rats stored on ice at 95 minutes following sampling.

6.3 Trial III, the effect of *in vitro* hemolysis on potassium ion concentration in bovine blood samples

Leakage of cell constituents into serum or plasma can lead to falsely elevated levels of various analytes among this potassium. Platelets have been shown to increase serum potassium levels by 0.36 ± 0.18 mmol compared to plasma samples due to the release of potassium in the course of the clotting process (Asirvatham et al., 2013). Hemolysis also has been shown to result in falsely elevation of plasma and serum potassium concentrations due to contamination of serum and plasma by intracellular constituents. Since 98% of body potassium is located intracellular, a small release of potassium can significantly affect the concentration of measured (extracellular) potassium (Asirvatham et al., 2013). The ratio between intracellular and extracellular potassium is approximately 40:1 and a change in the ratio of 2.5% would increase the potassium concentration by 0.1 mmol/l. Hemolysis is the most common cause of sample rejection by certified laboratories, as indicated by the College of American Pathologists (Jones et al., 1997). Mechanisms of interference from hemolysis have been summarized by Dimeski (2008) as follows: 1) Additive: released intracellular components e.g. K. LDH. AST 2) spectral: most notably at wavelength of 415, 540, and 570 nm where hemoglobin shows strong absorbance peaks 3) chemical: cross-reaction by free hemoglobin or other cellular constituents with the analyte of interest; 4) dilutional: intracellular fluid contamination in plasma and serum seen in severe hemolysis e.g. with sodium and chloride. As a consequence misinterpretation of results delivered by the laboratory occurs in case of pseudohyperkalemia or whenever potassium concentrations in blood from patients with hypokalemia are sited within the reference range due to hemolysis. The latter fact is even reflected in the reference ranges reported for serum and plasma potassium concentrations by various laboratories which has urged scientists to perform studies on reference ranges based on samples that were obtained under consideration of a proper pre-analytical procedure (Bertoni and Trevisi, 2013). Certified laboratories, however, have a quality control step for posted samples, which includes testing for the grade of hemolysis. Whenever hemolysis exceeds a pre-set grade - as determined by analysis of free hemoglobin in the serum or plasma phase- the samples are rejected due to insufficient quality and no measurements will be performed because misinterpretation of the results could be the consequence (Dimeski, 2008).

In the majority of hemolyzed specimens the intracellular potassium has been shown to be released by mechanical forces. Most often the use of vacuum tubes, the aspiration of blood from a catheter or unsuitable transport conditions were reported to cause hemolysis (Yücel and Dalva, 1992). Plasma hemoglobin might be serving as measure of hemolysis due to the fact that it is an indicator of erythrocyte destruction. The assessment of the presence and the grade of hemolysis in serum and plasma samples by visual control and comparison with a color chart, that has long been applied in diagnostic laboratories has been found unreliable due to the fact that hemolysis was shown to be present in samples without any visible discoloration of the plasma (Lippi et al., 2006, Owens et al., 2005, Hawkins, 2002, Jay and Provasek, 1993, Yücel and Dalva, 1992). To this end cut-off values have been established above which the assay is considered as compromised and samples are rejected as unsuitable (Dimeski, 2008). The cut off value is up to the laboratory determination.

In the present study, the free plasma hemoglobin content was used as indicator for *in vitro* hemolysis and was utilized to calculate the corrected potassium content for any given hemolyzed specimen, this was in agreement with a previous study by Owens et al. (2005).

Our findings proved that a significant positive linear relationship existed between the change in potassium concentration and FPHgb from non-hemolyzed to hemolyzed specimens. These findings were in agreement with previous studies by Jay and Provasek (1993) and Yücel and Dalva (1992) who found a linear relationship between the hemoglobin content and potassium concentrations in hemolyzed specimens. In addition, on basis of our dataset we have established a correction factor for calculating potassium concentrations in hemolyzed samples which was 0.0025 (at 95% confidence interval, 0.0018 to 0.0031) x FPHgb and this formula was obtained from a simple linear regression using a best-fit line through zero where the line of best fit for correction factor on our plot with nearly all points falling above the line and with using 95% confidence interval adds greater margin of safety and makes it more reliable. This was in agreement with a previous study, on human blood samples, by Owens et al. (2005) who found a correction factor of 0.00319 (95% confidence interval, 0.00290- 0.00349) x FPHgb. Use of a correction factor makes sense in cases where samples are unretrievable or when serial samples were drawn from a catheter in an experimental setting. Some authors, however, think that such an approach is unsuitable due to the great influence of hemolysis on plasma and serum analytes

(Yücel and Dalva, 1992). With respect to samples obtained from cattle there is a further aspect that has still to be taken into consideration. Cattle have been shown to harbor either high, intermediate or low potassium erythrocytes (Christinaz and Schatzmann, 1972). To this end the amount of potassium released into the plasma or serum due to hemolysis could differ between individuals although hemoglobin values are the same.

6.4 Trial IV, the relationship between muscle and plasma potassium concentrations

Due to fact that most of the body potassium is located inside the cell and that potassium is involved in the regulation of the glucose metabolism and the acid-base equilibrium as well as its dependency from aldosterone, various authors conclude that plasma or serum potassium levels do not give an accurate reflection of the total body potassium content. To this end the potassium content in hemolysates was used in order to gain more accurate information on the body total potassium content. Hemolysates, however, were found not suitable as predictors for total body potassium content (Janowitz, 1990, Ladefoged and Hagen, 1988) due to the wide variety of values obtained from samples collected from healthy cows. Also, the lack of correlation between potassium, magnesium and zinc concentrations in erythrocytes and muscle tissue was observed in a study by Ladefoged and Hagen (1988). These authors concluded on basis of their findings that evaluation of total body mineral content cannot be based on mineral concentrations in hemolysates. The situation is even more complicated in cattle as erythrocytes from members of this species have been found to contain either a low, intermediate or high potassium content, which is genetically determined. The main reason for different potassium contents in erythrocytes was found to be the higher pump activity and different density of pump sites in high potassium cells (Gustin et al., 1988).

Muscle biopsies could serve as indicator for body potassium content as proposed by various authors as the striated muscle has been shown to play a major role in maintenance of potassium homeostasis. The selection of the skeletal muscle in the present study was due to the fact that, skeletal muscle is considered the most sensitive and specific tissue for assessing whole body potassium and this was previously studied by Constable et al. (2014) and Palmer (2014) who stated that skeletal muscle contains approximately 75% of the whole body stores of potassium. At fasting and exercise the extracellular potassium pool is replenished from the muscles and after a

meal, the kidney and the muscles take part in elimination of potassium from the circulation. These mechanisms are under hormonal control and to this end are vulnerable for external influences. Trying to increase the understanding of potassium homeostasis in the post partum cow requires studies on the regulation of potassium distribution between the intracellular and extracellular space also termed internal potassium balance (Palmer, 2014). Obtaining muscle biopsies from animals that had to be euthanized for various reasons immediately following euthanasia was applied to gain first insights into the methodology and relationship between potassium contents in striated muscles and potassium concentrations in plasma of the same animal. Muscle biopsies are widely used in sports medicine and are also applied in the horse to examine exercise intolerance (Larsen et al., 1996, Johnson et al., 1991), but have only sporadically been applied in cattle (Constable et al., 2014).

The animals were euthanized using intravenous barbiturate which could have affected the quality of the biopsy material. Overdose of intravenous pentobarbital induces unconsciousness within seconds followed by respiratory depression and arrest, cessation of the heart beats, brain failure and death. Due to the fact that, most of the chemical methods used for euthanasia had significant effects on the measured metabolites and the presence of many other factors associated with euthanasia which can influence variability of the measurements; including handling of animals, site and timing of samples (Bhathena, 1992), to this end, we collected the muscle samples in the present study immediately after euthanasia and from the skeletal muscle of the hind quarter of animal (vastus lateralis muscle) to lower the influence of absorbed barbiturates on muscle biopsies measurements (Grieves et al., 2008, Port et al., 1978). The muscle biopsy technique as described by Bergström (1975) was applied by using a modified Bergström needle to obtain samples from the vastus lateralis muscle from the study animal.

The present study revealed that retrieving muscle biopsy samples was feasible when a modified Bergström needle was used (6 mm diameter, 100 mm length). Earlier studies at the Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin had shown that, the material obtained by biopsy technique was identified as pure muscle tissue as determined by visual observation followed by histological examination. The biopsy material did not demonstrate substantial amounts of connective tissue or fat tissue in the sample. Our results showed non-significant very weak correlation between potassium and sodium concentrations in plasma and

muscle samples. These findings were in agreement with previous investigations on the correlation between plasma or serum potassium concentrations and muscle tissue potassium contents in various animal species and man which revealed weak or no correlation. Previous studies on humans by Speich et al. (1987) proved that the plasma concentration do not necessarily reflect the total body stores of potassium, as low plasma potassium concentrations were observed in patients with normal intracellular potassium levels. Abraham and Lubran (1981), Lim and Jacob (1972), found marked deficiencies in the intracellular potassium content with no changes in plasma values.

In a previous study by Ladefoged and Hagen (1988) in 93 humans with low and normal plasma potassium concentrations the results showed no correlation between potassium concentrations in plasma, erythrocytes and muscles. Similar results were reported by Christinaz and Schatzmann (1972) who observed only a weak correlation between serum potassium concentrations and muscle potassium contents. No correlations were found between the plasma potassium and middle gluteal muscle potassium concentrations in 16 healthy adult horses according to a study by Palmer (2014).

Repeated intramuscular injections of isoflupredone acetate in cattle with ketosis resulted in downer cows due to hypokalemia. When isoflupredone acetate and furosemide were administered to multiparous Holstein Friesian cows in early lactation in an experiment these resulted in a decrease in plasma potassium concentrations in all three study groups, but - at the same time - only cows of a single group revealed a decrease in skeletal muscle potassium contents which indicates whole body potassium depletion as a response to treatment (Constable et al., 2014).

In the present study, there was a correlation observed between pH-values and plasma potassium concentrations while no correlation was observed between pH-values and muscle potassium content, the observed results indicate that the correlation between pH-values and the plasma potassium concentration is independent from changes in muscle potassium content in the same animal. Our results are in agreement with a previous studies by Bertoni and Trevisi (2013) on metabolic profiles in dairy cows who found that, acidosis increases the serum potassium concentration in cattle and alkalosis decreases but not necessarily affect the intracellular potassium content unless extreme changes in potassium levels occurred. In our study, the limited

numbers of samples included with only three blood samples with low pH-values do not allow conclusions with respect to the interplay between acid-base metabolism and muscle potassium content.

7 Conclusions

From the present studies the following conclusions can be drawn:

1. The degree of agreement found for direct ISE (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark) and FAES (AAS, SOLAAR M6, Thermo Fisher Scientific, Cambridge, United Kingdom) was found suitable to use ISE as a fast and reliable method for use in the animal hospital for determination of sodium and potassium in bovine emergency patients.

2. Direct ISE systems provide a more accurate estimation of plasma potassium and sodium ion concentrations in samples with hyper- as well as hypoproteinemia and hypercholesterolemia than FAES and for this reason should be used for clinical decision-making in sick animals.

3. Laboratories using indirect photometric methodology and indirect ISE to measure electrolyte status should take into account that abnormal plasma total protein and cholesterol concentrations could affect the results.

4. It was verified that the storage of blood samples obtained from cattle on ice does not demonstrate significant interference for blood gas analysis, glucose and sodium ion concentrations until 72 hours and for potassium ion concentrations until 48 hours following sampling. These findings are related to metabolic considerations and not to conclusions with respect to respiratory functions of the animal that require sampling of arterial blood. Although, it was evinced that low temperature is important to conserve viable samples stored, the importance of performing the blood analysis as soon as possible must not be excluded. The shortest time between blood collection and analysis in addition to storage of the blood samples on ice are purposing an ideal analysis.

5. The increase in plasma potassium concentration in hemolyzed specimens shows a linear relationship to plasma hemoglobin. This linear relationship between FPHgb and plasma potassium offers a useful correction factor of 0.0025 x FPHgb and at 95% confidence interval was (0.0018 to 0.0031) which can be applied whenever hemolytic samples are delivered to the laboratory which cannot be regained. If FPHgb was found to be significant, samples should be

rejected or reporting the results should be accompanied by a comment stating the FPHgb as well as the correction factor.

6. The Bergström technique to obtain muscle biopsies was applied on animals immediately after euthanasia. The concentration of potassium in muscle biopsy samples was only weakly correlated to that of the plasma. This result needs further investigations in a broad scale study on a greater number of animals.

8 Summary

Heba El-Zahar (2015):

Laboratory analysis of disorders in sodium and potassium homeostasis in cattle

Laboratory diagnosis of disorders in sodium or potassium homeostasis is affected by various factors among these the diagnostic method applied in the laboratory and the pre-analytical process. The sodium and potassium contents in plasma or serum can be determined by direct ISE "which requires no dilution step" and by indirect ISE or flame photometry, the latter techniques requiring a dilution step which influences the results. Errors in sodium and potassium measurements can lead to misinterpretation by the clinician. The present thesis should contribute to the knowledge on interferences that could cause alterations in the results of potassium and sodium measurements in bovine blood samples. To this end, the aims of the present study were as follows:

1. To determine the degree of agreement between results of measurements of potassium and sodium in diluted plasma and whole blood samples from cattle by FAES (AAS, SOLAAR M6, Thermo Fisher Scientific, Cambridge, United Kingdom) and ISE (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark).

2. To investigate the effect of selected analytes (total protein, cholesterol and pH) when lying outside the reference range on the results of sodium and potassium analysis (Trial I).

3. To examine the influence of the pre-analytical process (storage duration and temperature (Trial II) as well as hemolysis (Trial III) on analysis of potassium and sodium.

4. A first step towards determination of the intracellular potassium contents in diseased cattle was taken by comparison of potassium levels in blood with those in muscle biopsies in order to examine the relationship between the potassium ion concentrations in plasma and in muscle tissue (Trial IV).

In the first study a total of 26 blood samples collected from German Holstein cattle with laboratory values within the reference range were included for the agreement analysis between

ISE (EML™ 105) and FAES (AAS, SOLAAR M6). The study using Bland and Altman statistical method demonstrated a considerable agreement in the measurement of sodium and potassium by the two analyzers which fulfill the requirements of the IFCC and CLSI. The mean difference between the two analyzers was -2.9±2.6 and -0.04±0.11 for sodium and potassium, respectively. The values of sodium and potassium sited between the upper and lower limits of agreement at 95% confidence interval in samples within the reference range. This means that both analyzers can be used interchangeably.

Total protein levels below the reference range tended to cause a pseudohyper- effect for potassium and sodium when FAES was applied whereas high total protein levels resulted in a pseudohypo- effect but the effect was more prominent for sodium. In addition, globulins were more likely shown to cause a pseudohypo- and pseudohyper- effect than albumin.

The effect of storage time and temperature on potassium and sodium ion concentrations was studied on whole blood samples from cattle stored at room temperature (22^{o}C) and at 4^{o}C with time interval up to 72 hours. Potassium ion concentrations showed a significant increase in samples stored at room temperature (22^{o}C) (4.88±0.11 mmol/l) and at 4^{o}C (5.82±0.23 mmol/l) at 24 hours and 72 hours following sampling, respectively, compared to the initial measurements (3.84±0.11 mmol/l). Sodium ion concentrations in samples kept at 22^{o}C decreased significantly at 48 hours (139.6±0.42 mmol/l) while those kept at 4^{o}C showed no significant differences up to 72 hours compared to the initial measurements (141.68±0.49 mmol/l). The sodium-potassium pump is implicated in increasing potassium and decreasing sodium concentrations stored at both storage temperatures. At room temperature (22°C) the pump is activated due to increased production and utilization of ATPase, followed by a decreasing activity later on when glucose is decreased thus sodium enters the cells via diffusion and potassium leaves the cell by the concentration gradient. Our results showed a significant reduction in pH-values and glucose levels for samples kept at room temperature (22^{o}C) at 24 hours from sampling, with significant decreases in HCO_3^- concentrations at 48 hours. In parallel, the blood lactate levels significantly increased at 24 hours. The concentration of pCO_2 did not show any significant differences in samples kept at room temperature (22^{o}C) and 4^{o}C. The changes of blood gases and glucose in samples kept at 4^{o}C were not significantly up to 72 hours, while, blood lactate was significantly increased at 48 hours of the initial measurement. The continuing metabolic processes in the blood cause an increase in the

generation of CO_2 via the aerobic metabolism. After oxygen supply has ceased, lactate is formed through the process of anaerobic glycolysis which decrease the pH-value and HCO_3^- resulting in net cellular loss of potassium. At 4^oC storage temperature, minimal alterations were observed due to the slowdown of the cellular metabolism which diminished at low temperature.

Finally, it is concluded that the blood storage on ice does not modify the results of blood gas analysis, glucose and sodium ion concentration until 72 hours and potassium ion concentrations until 48 hours following sampling. Although, it was evinced that low temperature is important to conserve viable samples stored, the importance of performing the blood analysis as soon as possible or centrifugation of blood samples must not be excluded.

The effect of *in vitro* hemolysis on potassium concentrations was investigated in twenty bovine blood samples. The hemolysis was induced by mechanical manipulation by ejection of the blood immediately after collection into a vacuum tube with narrow needle. The potassium values become falsely elevated in hemolyzed samples where, the concentration of potassium in non-hemolyzed and hemolyzed samples has a mean value of 4.99±1.14 mmol/l and 5.24±1.18 mmol/l, respectively. The differences observed for potassium concentrations between hemolyzed (groups II and IV) and non-hemolyzed specimens (groups I and III) were 0.24±0.21 mmol/l and the plasma hemoglobin content in hemolyzed specimens was 82.77±68.1 mmol/l. A reliable correction factor to correct for falsely elevated potassium should be suitable in hemolyzed samples which are irretrievable. Where a high significant positive linear relationship between the change in potassium values and FPHgb from the non-hemolyzed to hemolyzed specimens existed; which offers a useful correction factor for potassium of 0.0025 (at 95% confidence interval, 0.0018 to 0.0031) x FPHgb in mg/dl. The correction factor can be applied for hemolyzed samples admitted to the laboratory, which incapable of being regained. If the FPHgb was significant and within the laboratory cut-off value of hemolysis, a comment would accompany the K^+ results including the FPHgb results in addition to the correction factor to advise clinicians more meaningfully. We suggest that, when the lower bound of the predicted potassium results in a corrected value within the reference range, a second blood draw will be unnecessary.

Muscle tissue is considered of greater value as indicator for body potassium status as plasma samples or hemolysates. Blood and muscle biopsy samples from 13 German Holstein cattle were collected for determining the relationship between plasma and muscle potassium contents. The potassium concentrations in both plasma and muscle biopsy samples were 4.01±0.72 mmol/l and 89.59±11.87 mmol/kg wet weight, respectively. A very weak correlation between the potassium and sodium concentrations in plasma and those in muscle samples was observed. In addition, the pH-values was correlated to plasma potassium concentration but not correlated to muscle potassium content. The observed results indicate that the correlation between pH-values and plasma potassium concentration was independent from the changes in muscle potassium content in the same animal due to the fact that, acidosis increases the serum potassium concentration in cattle and alkalosis decreases but not necessary affect the intracellular potassium content unless the extreme changes in potassium level occurred. For this, the evaluation of total body potassium content cannot be based on potassium concentrations in blood samples, due to fact that most of the body potassium is located inside the cell.

9 Zusammenfassung

Heba El-Zahar (2015):
Betrag zur Labordiagnostik von Störungen des Natrium- und Kaliumhaushaltes beim Rind

Die Labordiagnostik von Störungen des Natrium- und Kaliumhomöostase wird durch verschiedene Faktoren beeinflusst. Diese betreffen scoutet die Analyse im Labor, als auch die Präanalytik. Natrium- und Kaliumkonzentrationenim können im Vollblut, Plasma- und Serumproben mittels Ionen-selektiver Elektroden (ISE-Technologie) bestimmt werden. Während das direkte Verfahren keinen vorgeschalteten verdünnung-schnitt benötigt, geht der proben bestimmung beim indirekteren Verfahren ein Verdünnungs-schnitt voraus, durch den die Untersuchungs ergebnisse beeinflusst werden können. Fehler bei der Analytik von Natrium und Kalium können zu Missinterpretationen durch den Kliniker führen. Die vorliegende Studie soll einen Beitrag zum besseren Verständnis möglicher Störfaktoren leisten, die die Ergebnisse der Bestimmung von Natrium und Kalium in Blutproben vom Rind beeinflussen können. Die Ziele der vorliegenden Studie waren wie folgt:

1.) Ermittlung der Übereinstimmung der Ergebnisse der Analysen von Natrium und Kalium in verdünntem Plasma und Vollblutproben zwischen den Verfahren FAES (AAS, SOLAAR M6, Thermo Fisher Scientific, Cambridge, United Kingdom) und ISE (EML™ 105, Radiometer Medical A/S, Copenhagen, Denmark).

2.) Der Einfluss ausgewählter Parameter (Gesamtprotein, Cholesterin und pH-Wert) auf die Ergebnisse der Natrium- und Kalium-Analyse sollte untersucht werden, wobei Blutproben in denen die genannten Parameteraußerhalb ihres Referenzbereiches lagen, besondere Beachtung geschenkt wurde. (Test I)

3.) Untersuchung des Einflusses der präanalytischen Prozesse (Lagerungsdauer und Temperatur (Test II), sowie Hämolyse (Test III)) auf die Ergebnisse der Natrium- und Kaliumanalyse.

4.) Ein erster Schritt zur Bestimmung des intrazellulären Kaliumgehaltes in Muskelbiopsieproben sollte unternommen werden, um das Verhältnis der Kaliumkonzentration im Plasma und im Muskelgewebe (Test IV) zu ermitteln.

Im ersten Abschnitt wurden insgesamt 26 Blutproben von Rindern der Rasse Deutsch Holstein ausgewählt, deren Laborwerte im Referenzbereich lagen. An ihnen wurde die Übereinstimmung der Ergebnisse zwischen den Verfahren ISE (EML™ 105) und FAES (AAS, SOLAAR M6) bestimmt. Die statistischeAnalyse mittels der Bland-Altman Methode zeigte eine Übereinstimmung der Natrium- und Kaliumergebnisse zwischen den beiden Analyseverfahren, die den Anforderungen des IFCC und CLSI entsprechen. Die mittlere Differenz zwischen den beiden Verfahren waren für Natrium und Kalium -2,9±2,6 bzw. -0,04±0,11 mmol/l. Die Natrium- und Kaliumwerte lagen zwischen der oberen und der unteren Grenze Übereineinstimmung, was bedeutet, dass beide Analyseverfahren bei Probenmaterial innerhalb des Referenzbereiches austauschbar verwendet werden können.

Im zweiten Abschnitt führten Gesamtproteinwerte unterhalb des Referenzbereiches zu einem Pseudohypereffekt auf die Natrium- und Kaliumwerte beim FAES-Verfahren. Hohe Gesamtproteinwerte führten dagegen zu einem Pseudohypoeffekt, was ganz besonders für die Natriumwerte galt. Außerdem zeigten Globuline stärkere Pseudohypereffekt und Pseudohypoeffekt als Albumin.

Die Auswirkung der Lagerungsdauer und der Temperatur auf die Natrium- und Kaliumionenkonzentrationen wurde an den Rindervollblutproben bestimmt, die bei Raumtemperatur (22°C) und Kühlung (4°C) während eines Zeitraumes bis zu 72 Stunden gelagert wurden. DieKaliumionenkonzentrationen stiegen innerhalb von 24 und 72 Stunden in den Proben signifikant gegenüber der anfänglichen Messung an (3,84±0,11 mmol/l), wenn sie bei Raumtemperatur (22°C) (4,88±0,11 mmol/l) undgekühlt bei 4^{o}C (5,82±0,23 mmol/l) gelagert wurden.

Die Natriumionenkonzentrationen sanken signifikant nach 48 Stunden Lagerung bei 22°C Raumtemperatur ab (139,6±0,42 mmol/l), während die bei 4 °C gelagerten Proben, selbst nach 72 Stunden keinen signifikanten Unterschied zur Initialmessung (141,68±0,49 mmol/l) aufwiesen. Die Lagerungsbedingungen beeinflussen die Funktion der Natriumkaliumpumpe sodass Kalium- und die Natriumionenkonzentration bei beiden Lagerungsbedingungen zur Folge. Bei Raumtemperatur (22°C) ist die Pumpe zunächstaufgrund der erhöhten Aktivität der ATPase aktiviert. Später sinkt die Aktivität durch die abnehmende Glukosekonzentration, sodass Natrium

per Diffusion in die Zelle eindringt und Kalium aufgrund eines Konzentrationsgradienten aus der Zelle hinausdiffundiert. Unsere Ergebnisse zeigen ein signifikantes Absinken des pH- und des Glukosegehaltes bei Raumtemperatur (22°C) gelagerten Proben 24 Stunden nach der Entnahme und einen Anstieg der HCO_3^- Konzentration nach 48 Stunden. Parallel dazu steigen die Blutlaktatwerte signifikant nach 24 Stunden an. Die Konzentration von pCO_2 zeigte keine signifikanten Unterschiede in den Proben, die bei Raumtemperatur (22°C) und bei 4°C gelagert wurden. Die Veränderungen der Blutgase und der Glukosegehaltes bei den bei 4°C gelagerten Proben waren nicht signifikant bis zum Zeitpunkt von 72 Stunden, wohingegen die Laktatwerte signifikant 48 Stunden nach der Initialmessung anstiegen. Die anhaltenden Stoffwechselprozesse im Blut führten über den aeroben Stoffwechsel zu einem Anstieg von CO_2. Nachdem die Sauerstoffversorgung nicht mehr gewährleistet war, wurde über die anaerobe Glykolyse Laktat gebildet, welches zu einem Absinken des pH-Wertes führte und durch HCO_3^- in einem Nettoverlust von zellulärem Kalium endete. Bei 4°C Lagerungstemperatur wurden nur minimale Änderungen beobachtet, welche auf den verringerten Zellstoffwechsel bei der niedrigen Temperatur zurückzuführen sind.

Abschließend zeigt sich, dass die Lagerung von Blutproben auf Eis zu keiner signifikanten Änderung der Ergebnisse der Blutgasanalyse, der Glukosebestimmung und der Natriumionenkonzentration bis zu 72 Stunden, und der Kaliumionenkonzentration bis 48 Stunden nach der Initialmessung führt. Obwohl nachgewiesen wurde, dass niedrige Temperaturen für eine Konservierung der Proben sorgen, darf die Bedeutung der schnellen Durchführung der Blutanalyse bzw. des Zentrifugierens der Probe nicht außer Acht gelassen werden.

Die Wirkung der *in vitro* Hämolyse auf die Kaliumkonzentrationen wurde bei zwanzig Rinderblutproben untersucht.Die Hämolyse wurde,sofort nach der Blutentnahme durch mechanische Manipulation mittels des Auswurfes des Blutes durch eine schmale Nadel in ein Vakuumröhrchen, induziert. Die Kaliumgehalte waren bei hämolytischen Proben, im Vergleich zum Ausgangsmaterial, fälschlich erhöht. Die Mittelwerte der Kaliumkonzentration in nicht hämolytischen und hämolytischen Proben betrugen 4,99±1,14 mmol/l gegenüber 5,24±1,18 mmol/l. Die beobachteten Unterschiede der Kaliumkonzentration zwischen den hämolytischen (Gruppe II und IV) und den nicht hämolytischen Proben (Gruppe I und III) betrugen 0,24±0,21 mmol/l und der Plasmahämoglobingehalt in hämolytischen Proben betrug 82,77±68,1 mmol/l.

Ein zuverlässiger Faktor zur Korrektur von fälschlich erhöhten Kalium in hämolytischen Proben wäre geeignet der Ausgangsgehalt, die unwiederbringlich sind zu bestimmen. Zwischen den Änderungen der Kaliumwerte und FPHgb von hämolytischen und nicht hämolytischen Proben existiert eine hoch signifikante positive lineare Beziehung, welche einen nutzbaren Korrekturfaktor für Kalium von 0,0025 (bei einem 95%igen Konfidenzintervall, 0.0018 bis 0.0031)xFPHgb, bringt.Der Korrekturfaktor kann beihämolytischenProben im Labor angewendet werden, bei denen eine erneute Probenentnahme nicht möglich ist. Wenn der FPHgb-Wert als signifikant befunden wird und unterhalb der Höchstgrenze des Hämolysewertes liegt, könnte ein Kommentar die Kaliumwerte zusammen mit den FPHgb-Ergebnissen und dem Korrekturfaktorbegleiten, um den Kliniker besser zu beraten. Wir schlagen vor, dass danneine zweite Blutentnahme überflüssig wird, wenn die Untergrenze des vorhergesagten Kaliumwertes beim korrigierten Wert innerhalb des Referenzbereiches liegt.

Muskelgewebe wird gegenüber Plasmaproben oder Hämolysaten, als bedeutenderer Indikator für den Körperkaliumstatus angesehen. Von 13 Rindern der Rasse Deutsche Holstein wurdenBlut- und Muskelbiopsieproben gesammelt, um die Beziehung zwischen dem Plasma- und dem Muskelkaliumgehalt zu bestimmen. Die Kaliumkonzentration im Plasma und in den Muskelbiopsieproben betrug 4,01±0,72 mmol/l und 89,59±11,87 mmol je kg Nassgewicht.Eine sehr schwache Korrelation wurde zwischen den Kalium- und Natriumkonzentrationen im Plasma und denen in den Muskelproben beobachtet. Des Weiterenwar der pH-Wert zwar mit der Plasmakaliumkonzentrationkorreliert, nicht aber mit dem Muskelkaliumgehalt.Die beobachteten Ergebnisse deuten darauf hin, dass die Korrelation zwischen dem pH-Wert und der Plasmakaliumkonzentrationunabhängig von den Veränderungen im Muskelkaliumgehalt beim selben Tier war. Aufgrund der Tatsache, dass eine Azidose die Serumkaliumkonzentration im Rind erhöht, und eine Alkalose dieselbe erniedrigt, müssen diese aber nicht zwingend den interzellulären Kaliumgehalt verändern, außer wenn extreme Veränderungen im Kaliumwert auftreten. Deshalb lässt die Kaliumkonzentration in Blutproben keine Aussage über den Kaliumstatus eines Lebewesens zu, da der größte Anteil des Körperkaliumgehaltes innerhalb der Zellen lokalisiert ist.

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11 Appendix

Table 27. List of the basic data of 26 cattle used in study I for performing an agreement analysis between FAES (AAS, SOLAAR M6) and ISE (EML™105) including age, breed, sex, diagnosis and the locality of the cattle

No.	Sex	Breed	Age (Years)	Diagnosis
1	Female	German Holstein	0.2	Umbalical hernia, bronchopneumonia
2	Female	German Holstein	0.2	Umbalical hernia, bronchopneumonia and conjunctivitis
3	Female	German Holstein	6.64	Ketosis, sole ulcer, pericarditis
4	Female	German Holstein	4.73	Tympany, vagal indigestion syndrome, mastitis
5	Female	German Holstein	-	LDA, secondary ketosis, chronic peritonitis, dermatitis
6	Female	German Holstein	-	LDA, secondary Ketosis, dermatitis, claw ulcer
7	Female	German Holstein	-	Abomasal atony, secundary ketosis, peritonitis, right abomasal displacement and abomasal ulcer
8	Female	German Holstein	5.25	Ketosis, Dermatitis, claw affection
9	Female	German Holstein	5.29	LDA, secondary ketosis, fatty liver syndrom
10	Female	German Holstein	3.37	Intestinal obstruction, Clostridiosis, hemorrahgic bowel syndrome
11	Female	German Holstein	3.62	Peritonitis
12	Female	German Holstein	3.72	RDA
13	Female	German Holstein	3.63	Peritonitis
14	Female	German Holstein	-	Healthy
15	Female	German Holstein	4.64	Healthy
16	Female	German Holstein	-	Healthy
17	Female	German Holstein	2.54	Healthy
18	Female	German Holstein	2.26	Healthy
19	Female	German Holstein	2.45	Healthy
20	Female	German Holstein	2.37	Healthy
21	Female	German Holstein	-	Healthy
22	Female	German Holstein	1.87	Healthy
23	Female	German Holstein	1.78	Healthy
24	Female	German Holstein	2.1	Healthy
25	Female	German Holstein	-	-
26	Female	German Holstein	3.88	LDA, Metritis

Table 28. The raw laboratory data of 26 plasma and whole blood samples from cattle used in the agreement analysis in study I, temperature, blood gases, potassium and sodium concentration measured by FAES (AAS, SOLAAR M6) and ISE (EML™ 105), total protein, cholesterol and water mass concentrations. Results of potassium and sodium measurements by FAES are expressed as total substance concentration and those by ISE are expressed as substance concentration

No.	Temp.	pH-values	CO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FP (mmol/l)	Potassium ISE (mmol/l)	Sodium FP (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
1	38.8	7.43	52	28	4	4.1	4	133.42	139	67.8	35.9	31.9	2.55	93.76
2	38	7.41	52	32	7	3.66	3.6	133.94	139	66.1	34.3	27	2.11	93.34
3	38	7.42	41	21	-1	3.71	3.9	139.8	143	79	35.5	43.5	2	93.266
4	38	7.47	39	28	3	3.83	4	131.39	139	77.15	32.2	44.95	4.28	92.518
5	38.9	7.42	53	21	-3	3.1	3.2	134.54	139	74.3	37.8	35.7	2.8	92.91
6	39	7.42	41	23	0	4.05	4	133.87	137	76.7	31.8	44.9	2.36	92.77
7	39.7	7.41	36	28	2	3.14	3.1	135.97	136	73.8	34.7	39.1	1.95	93.172
8	38.2	7.38	37	33	8	3.21	3.2	139.36	142	73.8	37.7	35.4	1.9	93.98
9	38.6	7.43	40	27	2	4.19	4.4	136.07	143	72.4	35.7	36.7	2.03	93
10	38.4	7.41	47	25	2	3.33	3.4	132.9	136	79.2	37.9	40.8	2.87	92.727
11	38.5	7.41	48	29	5	4.27	4.2	136.83	139	70.3	33.7	36.6	2.41	93.54
12	38.2	7.37	51	32	6	4.1	4.2	131.94	139	76	32.6	43.4	2.52	92.82

Table 28 continued

No.	Temp.	pH-values	CO_2 (mmHg)	HCO_3^- (mmol/l)	Base excess (mmol/l)	Potassium FP (mmol/l)	Potassium ISE (mmol/l)	Sodium FP (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
13	38.5	7.44	45	27	3	4.23	4.3	135.05	138	75.8	30.4	45.4	2.19	93.11
14	38.5	7.42	41	27	4	4.97	4.9	138.97	138	75.8	38	37.8	2.68	92.759
15	38.4	7.41	54	21	-2	4.57	4.4	142.81	139	69.4	35.3	34.1	2.77	93.023
16	38.4	7.42	44	23	0	4.6	4.6	140.04	140	75.05	35.1	39.95	2.23	92.761
17	38.6	7.4	47	26	2	4.96	4.9	138.46	141	72.6	35.7	36.9	2.19	92.654
18	38.6	7.45	41	27	3	3.72	3.8	137.27	140	73	36.3	36.7	2.69	93.026
19	38.5	7.38	37	30	6	4.27	4.3	140.83	142	66	34.7	27.4	1.99	93.002
20	38.4	7.43	36	28	3	4.1	4.2	138.12	140	69.1	37.2	31.9	2.37	92.679
21	38.6	7.37	49	33	8	4.77	4.7	140.46	140	65.9	37	28.9	2.38	92.656
22	38.4	7.4	45	27	4	4.32	4.5	137.32	140	77.2	37.3	36.9	2.09	92.88
23	38.6	7.42	45	26	2	4.09	4.2	135.47	140	74.5	37.9	33.6	2.87	93.23
24	38.6	7.45	45	30	7	4.21	4.3	134.59	138	80	38	42	3.98	93.436
25	38.7	7.4	54	29	5	4.49	4.7	138.09	142	77.3	36.7	40.6	4.63	93.26
26	37.9	7.41	37	25	2	4.19	4.4	131.63	137	74	35	39	1.92	92.778

Table 29. List of the basic data of 93 cattle used in study I to determine the relationship between the differences in sodium and potassium measurements by the two analyzers and changes in total protein, albumin, globulin, cholesterol concentrations and pH-values. For total protein, albumin, globulin samples from 1 to 24, for cholesterol samples from 25 to 63 and for pH samples from 64 to 93 were used

No.	Sex	Breed	Age (Years)	Diagnosis
1	Female	German Holstein	3.45	Paratuberculosis
2	Female	German Holstein	2.74	Mastitis
3	Female	German Holstein	4.99	Lung emphysem, thrombophlebitis and pneumonia
4	Female	German Holstein	6.24	Left displaced abomasum, secondary ketosis and peritonitis
5	Female	German Holstein		Neonatal diarrhae, bronchpneumonia
6	Female	German Holstein	3.28	Left displaced abomasum and metritis
7	Female	German Holstein	0.8	Sole ulcer, osteomyelitis and claw amputation
8	Female	German Holstein	0.98	Sole ulcer and osteomylitis
9	Female	German Holstein	4	Sole ulcer
10	Female	German Holstein	2.72	Sole ulcer, elbow abcess and gluteal muscle hematoma
11	Male	German Holstein	14 days	Enteritis
12	Female	German Holstein	8.3	Bursitis
13	Female	German Holstein	2.83	Aspiration pneumonia, Epistaxis and left intranasal neoplasia
14	Female	German Holstein	4.5	Mastitis and metritis
15	Female	Fleischrind x Milchrind	0.07	Enteritis and umblical hernia
16	Female	German Holstein	0.1	Bronchopnemonia
17	Female	German Holstein	0.2	Bronchopneumonia and umblical hernia
18	Female	German Holstein	2.45	Routine biochemistry analysis
19	Female	German Holstein	2.55	Routine biochemistry analysis
20	Female	German Holstein		Left displaced abomasum and metritis
21	Female	German Holstein	5.3	Purulent tendovaginitis
22	Female	German Holstein	5.4	Purulent tendovaginitis
23	Female	German Holstein		Routine biochemistry analysis
24	Female	German Holstein	11	Endocarditis and myocarditis
25	Female	German Holstein		Left displaced abomasum, metritis and thrombophlebitis
26	Female	German Holstein	3.8	Left displaced abomasum and secondary ketosis, abcess
27	Female	German Holstein	2.1	Left displaced abomasum and secondary ketosis
28	Female	German Holstein	5.6	Secondary ketosis and endometritis

Table 29 continued

No.	Sex	Breed	Age (Years)	Diagnosis
29	Female	German Holstein		Left displaced abomasum, metritis, peritonitis and fatty liver
30	Female	Red Holestien	8.97	Claw defect and dermatitis
31	Female	German Holstein	0.1	Bronchopneumonia and umblical hernia
32	Female	German Holstein		Subclinical mastitis
33	Female	German Holstein	2.5	Peritonitis and interabdominal abcess
34	Female	German Holstein		Left displaced abomasum and secoundry ketosis
35	Female	German Holstein	2.88	Claw amputation with digital dermatitis
36	Female	German Holstein	3.71	Right abomasal displacement
37	Female	German Holstein	2.8	Claw defect
38	Female	German Holstein	5.56	Mastitis
39	Female	German Holstein	2.3	Abcess
40	Female	German Holstein	2.3	Abcess
41	Female	German Holstein	8.55	Secoundary ketosis, bronchopneumonia and fatty liver
42	Female	German Holstein	0.3	Bronchopneumonia and umblical hernia
43	Female	German Holstein	3.65	Peritonitis
44	Female	German Holstein	3.5	Enteritis
45	Female	German Holstein	5.44	Routine biochemistry analysis
46	Female	German Holstein	2.57	Routine biochemistry analysis
47	Female	German Holstein	2.6	Routine biochemistry analysis
48	Female	German Holstein	2.5	Routine biochemistry analysis
49	Female	German Holstein	4.8	Routine biochemistry analysis
50	Female	German Holstein	2.5	Routine biochemistry analysis
51	Female	German Holstein	4.87	Routine biochemistry analysis
52	Female	German Holstein	5	Left displaced abomasum, secondary ketosis, fatty liver and abomasal ulcer
53	Male	German Holstein	1.45	Routine biochemistry analysis
54	Female	German Holstein	5.67	Routine biochemistry analysis
55	Female	Fleischrind x Milchrind	1.38	Routine biochemistry analysis
56	Female	German Holstein	3	Routine biochemistry analysis
57	Female	German Holstein	3.21	Routine biochemistry analysis
58	Female	German Holstein	4.3	Left displaced abomasum and secondary ketosis
59	Female	German Holstein	0.54	Bronchitis
60	Female	German Holstein		Routine biochemistry analysis
61	Female	German Holstein		Routine biochemistry analysis

Table 29 continued

No.	Sex	Breed	Age (Years)	Diagnosis
62	Female	German Holstein		Routine biochemistry analysis
63	Female	German Holstein	3.27	Left displaced abomasum, metritis and udder eczema
64	Female	German Holstein	3.8	Left displaced abomasum and secondary ketosis, abcess
65	Female	German Holstein	2.2	Dermatitis
66	Female	German Holstein	4.2	Thrombophlebitis of the right mammary vein
67	Female	German Holstein	4.77	Vagal indigestion, tympany and mastitis
68	Female	German Holstein	0.9	Claw ulcer and osteomylitis
69	Female	German Holstein		Left displaced abomasum, secondary ketosis and metritis
70	Female	German Holstein		Routine biochemistry analysis
71	Female	German Holstein	5	Claw ulcer and mastitis
72	Female	German Holstein	2.69	Claw affection
73	Female	German Holstein	0.11	Bronchopneumonia, umblical hernia and enteritis
74	Female	German Holstein	3	Left displaced abomasum
75	Female	German Holstein	2.58	Routine biochemistry analysis
76	Female	German Holstein	4.4	Routine biochemistry analysis
77	Female	German Holstein		Routine biochemistry analysis
78	Female	German Holstein		Routine biochemistry analysis
79	Female	German Holstein	4.55	Routine biochemistry analysis
80	Female	German Holstein	7.94	Retained placenta
81	Female	German Holstein	5	Routine biochemistry analysis
82	Female	German Holstein	7.17	Routine biochemistry analysis
83	Female	German Holstein		Routine biochemistry analysis
84	Female	German Holstein		Routine biochemistry analysis
85	Female	German Holstein	6.2	Vagal indigestion
86	Female	German Holstein	2.5	Peritonitis and interabdominal abcess
87	Female	Fleischrind	4.99	Aspiration pneumonia and pharyngeal obstruction
88	Female	Red Holstein	3.79	Secondary ketosis, fatty liver and lung emphysema
89	Female	German Holstein		Routine biochemistry analysis
90	Female	German Holstein	3.29	Left displaced abomasum and metritis
91	Castrated male	Gallowy	5.25	Routine biochemistry analysis
92	Female	German Holstein	6.87	Pneumonia, pericarditis and peritonitis
93	Female	German Holstein	6.6	Abomasal ulcer and chronic peritonitis

Table 30. The raw laboratory data of 24 plasma and whole blood samples from cattle used in study I, for investigating the relationship between the concentration of total protein, albumin and globulin and the differences of potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). The data include, temp., pH, pCO_2, HCO_3^-, Base Excess, potassium, sodium, total protein, albumin, globulin, cholesterol and water mass

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
1	38.1	7.45	42	28	4	4.45	4.4	135.9	134	59	26	33	1.74	92.8
2	39	7.42	52	32	8	4.4	4.4	135.23	138	96.64	30.14	66.5	2.79	91.94
3	38.5	7.3	37	17	-8	3.53	3.5	134.61	140	87.9	37.8	50.1	4.16	92.83
4	38.5	7.39	41	24	0	3.46	3.4	128.84	138	108.35	33.9	74.45	1.87	90.86
5	38.4	7.36	66	36	8	5.59	5.5	145.2	140	55.5	32.7	22.8	1.98	93.1
6	38.2	7.4	47	28	4	3.24	3.2	139.09	146	55.3	28	27.3	1.47	94.17
7	38.3	7.38	39	22	-2	4.15	4.2	139.43	142	83.1	36.7	46.4	3.87	92.68
8	38	7.45	44	30	6	4.19	4.5	132.66	141	87	33.3	53.7	2.17	91.99
9	38.2	7.4	47	28	3	4.7	4.6	135.61	139	98	33.9	64.1	0.04	93.9
10	38.7	7.38	47	27	2	3.85	3.9	130.45	140	104.2	30.7	73.5	2.33	91.4
11	38.5	7.34	51	26	0	4.41	4.4	133.99	135	64.5	37.2	27.3	2.3	95.5

Table 30 continued

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
12	37.9	7.38	38	21	-2	4.55	4.6	133.29	138	81.7	36	45.7	3.24	89.5
13	35.9	7.49	41	32	7	4.18	4.3	137.32	140	97.4	37	60.4	4.45	93.6
14	38.4	7.29	41	19	-7	4.62	4.8	126.38	128	97.6	30.2	67.4	1.95	89.2
15	38.8	7.4	57	34	8	4.36	4.3	133.85	137	48.7	35.6	13.1	1.9	96.68
16	38.5	7.36	49	27	2	3.28	3.2	130.38	136	62.7	34.2	28.5	2.97	93.41
17	38.1	7.34	52	27	1	4.27	4.1	137.52	140	62.7	38.9	23.8	1.92	95.51
18	38.6	7.36	51	27	2	4.17	4.2	131.29	139	66.4	35.7	30.7	2.8	92.7
19	38.5	7.36	54	29	4	4.2	4.2	141.34	140	70.25	35.1	35.15	2.67	92.5
20	38.7	7.4	41	24	1	4.27	4.2	141.1	138	60.7	30.7	30	0.73	92.5
21	38.2	7.43	43	28	4	4.11	4.1	134.15	140	92.25	31.9	60.35	4.2	90.02
22	38.2	7.45	43	28	5	4.62	4.6	126.89	139	98.2	32.3	65.9	4.05	89.94
23	38.8	7.3	61	28	1	4.54	4.6	140.96	142	74.6	39.7	34.9	4.49	93.59
24	38.5	7.48	29	21	0	3.88	4.1	130.68	137	98.6	30.3	68.3	2.42	92.66

Table 31 The raw laboratory data of 39 plasma and whole blood samples from cattle used in study I, for investigating the relationship between the concentration of cholesterol and the differences of potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). The data include, temp., pH, pCO_2, HCO_3^-, Base Excess, potassium, sodium, total protein, albumin, globulin, cholesterol and water mass

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
25	39	7.3	31	14	-10	4.76	4.7	135.18	136	70.76	30.37	40.39	1.33	95.39
26	39.1	7.41	41	25	2	3.94	3.9	136.33	137	81.8	37.07	44.73	2.49	95.13
27	39.9	7.33	36	18	-6	2.6	2.6	135.75	132	78.1	36.1	42	1.36	90
28	39.9	7.36	53	28	3	4.32	4.4	137.55	142	80.8	32.9	47.9	2.87	93.2
29	38.7	7.37	58	32	5	3.83	3.8	142.65	143	66	30.6	35.4	0.83	93.22
30	38.9	7.27	71	31	3	5.11	5.1	136.75	139	81.6	40	41.6	4.64	92.73
31	38	7.39	55	32	7	3.7	3.6	137.92	139	62.2	35.2	27	1.82	96.73
32	39.1	7.34	38	20	-4	4.2	4.3	124.23	137	83.5	30.1	53.4	2.05	93.99
33	39.9	7.34	52	26	1	4.63	4.6	133.76	138	73.5	28.5	45	2.36	92.1
34	38.6	7.37	38	21	-3	4.23	4.2	134.63	138	71.05	36.9	34.15	1.16	94
35	38.5	7.42	43	27	4	4.24	4.2	140.39	137	91.6	39.35	52.25	0.12	90.1
36	37.9	7.26	35	15	-11	4.11	5.1	136.71	135	70.7	33.8	36.9	2.75	95.3

Table 31 continued

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
37	38.6	7.33	52	27	1	4.44	4.6	132.89	141	83.6	38.5	45.1	4.85	88.8
38	40.2	7.44	37	24	2	3.61	3.8	125.79	134	84.7	30.3	54.4	2.75	90
39	38.1	7.42	43	27	3	4.02	4.2	129.35	138	73.1	35.9	37.2	5.53	92.79
40	38.3	7.41	44	27	2	4.55	4.6	134.87	137	78.3	36.7	41.6	6	91.6
41	38.1	7.24	38	15	-11	2.71	2.7	133.01	139	69.3	37	32.3	1.84	94.78
42	38.5	7.36	49	26	1	4.13	4.3	134.78	140	66.9	37.4	29.5	1.93	92.7
43	38.4	7.43	44	28	4	4.32	4.4	133.59	138	81	28.1	52.9	2.18	93.95
44	38.9	7.47	45	30	7	3.81	4.1	131.75	139	85.2	38.6	46.6	6.8	91.29
45	38.6	7.36	46	25	0	4.62	4.7	132.8	136	71.5	33.5	38	2.22	92.8
46	38.4	7.39	50	29	4	4.89	4.8	141.49	138	73.4	37.9	35.5	5.03	93.81
47	38.4	7.39	50	29	4	5.05	4.8	140.88	140	75.1	38.2	36.9	4.94	94.73
48	38.5	7.43	43	28	4	4.23	4.2	136.79	137	77.4	39.4	38	6.1	94.32
49	38.5	7.34	53	27	1	5.19	5.3	136.03	139	79	41.4	37.6	6.2	92.81
50	38.6	7.36	58	31	5	4.79	4.7	138.64	139	79.7	39.5	40.2	6.97	93.12

Table 31 continued

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
51	38.6	7.35	51	27	2	5.23	5.1	141.03	141	81.3	40.6	40.7	5.35	93.46
52	38.6	7.36	37	20	-4	3.94	3.8	144.27	139	66.2	34.3	31.9	0.98	92.53
53	38.6	7.33	60	30	4	4.79	4.7	135.99	138	66.2	35	31.2	2.12	94.6
54	38.5	7.37	52	29	4	4.71	4.7	135.04	138	67.9	39.3	28.6	0.02	91.6
55	38.6	7.34	58	30	4	4.94	4.8	139.89	140	68.3	37.1	31.2	3.3	92.7
56	38.6	7.35	59	31	5	4.22	3.9	137.26	142	68.8	37.7	31.1	0.03	93.5
57	38.5	7.33	60	30	3	5.14	5	135.74	140	78.6	41.25	37.35	0.005	94.3
58	38.1	7.32	39	19	-5	3.38	3.3	137.83	137	69.9	36.08	33.82	3.7	95.34
59	38.6	7.36	51	27	2	3.64	3.6	134.93	137	64.4	34.7	29.7	1.67	93.4
60	38.3	7.34	53	27	2	4.75	4.9	137.91	140	70.7	37.1	33.6	3.53	91.45
61	38.7	7.42	46	28	4	4.16	4.2	140.65	142	76.3	37.4	38.9	6	97.09
62	38.1	7.31	70	33	5	4.29	4.7	141.33	146	78.5	37.5	41	5.77	94.79
63	38.5	7.35	39	20	-4	4.01	4	134.54	138	74.8	33	41.8	1.65	93.4

Table 32 The raw laboratory data of 30 plasma and whole blood samples from cattle used in study I, for investigating the relationship between blood pH-values and the differences of potassium and sodium measurements by FAES (AAS, SOLAAR M6) and ISE (EML™ 105). The data include, temp., pH, pCO_2, HCO_3^-, Base Excess, potassium, sodium, total protein, albumin, globulin, cholesterol and water mass

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
64	38.5	7.37	46	25	1	3.34	3.5	130.91	137	88.4	37.5	50.9	2.39	92.96
65	38.3	7.37	59	33	6	4.12	4.3	129.57	140	85.7	37.3	48.4	3.13	91.86
66	39.9	7.4	40	24	1	3.92	4	137.01	138	72	33.2	38.8	1	90.5
67	38.6	7.36	46	24	0	4.28	4.5	136.27	141	78.4	32.9	45.5	4.05	93.4
68	39.1	7.44	43	28	5	3.93	4	136.62	142	84.7	33	51.7	2.7	91.54
69	39	7.49	49	28	4	4.18	4.3	137.32	140	71.55	32.8	38.75	1.67	91.55
70	38.5	7.44	39	25	2	4.21	4.4	134.78	141	87.3	38.5	48.8	1.71	92.98
71	38.1	7.4	45	27	2	4.02	4.2	130.39	139	90.5	38.2	52.3	3.5	92.96
72	35.9	7.37	57	33	5	3.92	4	138.27	141	80.2	33.4	46.8	3.49	93.6
73	38.6	7.41	64	39	12	4.38	4.4	136.68	140	51.4	31.2	20.2	2.27	93.5
74	38.6	7.47	51	27	2	3.81	4.1	131.75	139	69.4	34.5	34.9	1.82	92.9
75	38.4	7.48	33	28	5	3.73	3.9	131.01	139	65.6	35.9	29.7	2.18	92.1
76	38.6	7.48	37	27	4	3.86	4	133.65	142	65.7	35.4	30.3	1.92	92.7
77	38.5	7.47	39	28	5	4.28	4.2	137.23	138	71.5	33.8	37.7	2.39	92.4
78	38.5	7.47	32	22	0	3.9	3.9	127.9	129	73.6	33.2	40.4	2.01	91.9

Table 32 continued

No.	Temp. °C	pH-values	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium FAES (mmol/l)	Potassium ISE (mmol/l)	Sodium FAES (mmol/l)	Sodium ISE (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Cholesterol (mmol/l)	Water mass (%)
79	38	7.34	56	29	3	4.71	4.8	132.18	139	78.9	35.7	43.2	3.22	91.1
80	39.1	7.43	37	22	-2	4.11	4.1	134.15	140	86.5	30.9	55.6	2.04	92.8
81	38.4	7.37	56	31	5	4.63	4.6	132.59	137	81.9	41	40.9	4.25	95.71
82	38.4	7.4	50	29	4	4.05	4.2	136.42	140	83.75	35	48.75	2.68	90.8
83	38.5	7.38	44	25	1	4.77	5	135.37	139	84.5	34.9	49.6	2.46	90.7
84	38.4	7.38	46	27	2	4.36	4.6	128.81	139	90.7	33.9	56.8	2.74	89.8
85	38	7.36	50	27	2	4.65	4.8	136.55	141	81.8	37.5	44.3	4.15	92.35
86	38.6	7.48	50	29	4	3.88	4.1	130.68	137	84.2	28.3	55.9	2.23	92.7
87	35.9	7.42	27	17	-6	4.61	4.5	140.79	133	83.4	38.7	44.7	2.71	93.5
88	39.9	7.14	34	10	-17	2.44	2.6	147.2	154	72.3	36.5	35.8	2.93	89.9
89	38.5	7.4	49	29	4	4.03	4.1	135.36	140	67.2	37.6	29.6	0.02	95.2
90	38.1	7.47	38	27	4	4.63	4.7	129.07	134	70.4	29.05	41.35	1.84	91.84
91	38.6	7.47	43	31	7	3.89	3.8	141.08	143	78.15	38.7	39.45	0.11	93.9
92	35.9	7.51	53	42	16	3.31	3.4	133.4	133	78.1	36.5	41.6	3.84	91.8
93	37.5	7.4	51	31	6	3.5	3.6	136.54	141	61.1	28.8	32.3	2.32	93.33

Table 33. The basic data of cattle used in study II, illustrating the basic information of cattle used in the study

No.	Breed	Sex	Age (Years)
1	German Holstein	Female	10.5
2	German Holstein	Female	2.9
3	German Holstein	Female	4.2
4	Fleischrind x Milchrind	Female	6.6
5	German Holstein	Female	12.6
6	German Holstein	Female	4
7	German Holstein	Female	6.6
8	German Holstein	Female	2.8
9	Fleischrind x Milchrind	Female	6
10	German Holstein	Female	2.5

Table 34. The basic and the raw laboratory data in study III including basic information of cattle, temp., pH, pCO_2, HCO_3^-, Base Excess and the potassium and sodium concentrations by ISE (EML™ 105)

No	Breed	Sex	Age (Years)	Temp. °C	pH-value	pCO_2 (mmHg)	HCO_3^- (mmol/l)	Base Excess (mmol/l)	Potassium concentration (mmol/l)	Sodium concentration (mmol/l)
1	Galloway	Female	7.7	38.1	7.42	33	20	-2	3.7	136
2	Galloway	Female	8.8	38.2	7.42	49	31	6	3.8	141
3	Galloway	Female	0.6	38.2	7.35	53	28	2	4	137
4	Galloway	Male	2.3	37.9	7.38	51	29	3	4.2	139
5	Galloway	Female	2.3	38.6	7.35	44	23	-1	4.2	137
6	Galloway	Female	5	38.3	7.32	53	26	0	4.4	139
7	Galloway	Male	6.3	38.7	7.37	47	26	1	4.4	137
8	Charolais	Female	0.8	38.1	7.29	60	28	0	4.6	137
9	Galloway	Female	0.97	38.8	7.33	49	25	-1	4.8	137
10	Galloway	Male	0.7	37.9	7.37	40	30	4	4.5	138
11	German Holstein	Female	-	38.5	7.38	45	26	1	4.3	141
12	German Holstein	Female	-	38.5	7.36	51	28	3	4.6	138
13	German Holstein	Female	-	38	7.35	45	24	-1	4.5	139
14	German Holstein	Female	-	38.6	7.35	59	31	4	3.9	137
15	German Holstein	Female	-	38.1	7.37	46	26	1	4.1	139
16	German Holstein	Female	-	38.6	7.44	39	25	2	4.3	139
17	German Holstein	Female	-	38.2	7.39	43	25	1	4	142
18	German Holstein	Female	-	38.5	7.39	46	27	2	4	138
19	German Holstein	Female	-	38.4	7.39	45	26	2	4	139
20	German Holstein	Female	-	38.7	7.35	53	28	2	4.4	137

Table 35. The basic data in muscle biopsy study IV, including age, sex, breed and the location of the patient

No.	Breed	Sex	Age	Temp.
1	Holstein Fleckvieh	Female	7 m.	38.4
2	German Holstein	Female	4 m.	38.6
3	German Holstein	Female	15 m.	38.5
4	German Holstein	Female	2 y	38.1
5	German Holstein	-	-	38.6
6	German Holstein	-	-	38.7
7	German Holstein	Female	3 m.	38.4
8	German Holstein	-	-	39
9	German Holstein	Female	3.3 m.	36.8
10	German Holstein	Female	6 m.	37.5
11	-	-	-	38.1
12	German Holstein	Male	1.3 y	38.4
13	Holstein Fleckvieh	Male	1 m.	38.9

Table 36. The raw data in muscle biopsy study IV, sodium and potassium concentrations were measured in both plasma and muscle biopsies. Results of potassium and sodium measurements in plasma by ISE are expressed as substance concentration

	Plasma analysis						Muscle biopsy analysis		
No.	**Sodium (mmol/l)**	**Potassium (mmol/l)**	**pH**	**pCO_2 (mmHg)**	**HCO_3^- (mmol/l)**	**Base Excess (mmol/l)**	**Sodium (mmol/kg)**	**Potassium (mmol/kg)**	**Water content (g/kg)**
1	140	3.6	7.39	49	28	3	67.75	98.71	247.04
2	134	3.8	7.38	45	26	1	54.24	80.35	230.02
3	136	4.6	7.33	59	29	3	43.10	80.26	240.81
4	141	4.2	7.43	45	29	5	54.72	93.63	271.34
5	138	4.7	7.36	55	29	4	62.61	105.16	248.34
6	136	4.2	7.42	49	31	6	51.92	86.80	213.79
7	129	2.8	7.4	47	28	4	97.18	104.27	198.573
8	139	4.4	7.38	51	29	4	84.17	108.93	245.927
9	135	4	7.42	47	30	5	120.01	90.41	241.609
10	134	2.5	7.2	48	18	-10	102.60	78.26	220.04
11	136	4.6	7.45	39	26	3	91.93	77.78	241.27
12	139	3.8	7.48	41	30	6	184.67	72.03	255.70
13	134	4.9	7.33	61	30	4	113.19	88.10	225.12

12 List of publications

1. **El-Zahar Heba**, Müller Kerstin (2013): Determination of sodium and potassium levels in bovine blood by ion-selective electrodes and atomic emission spectroscopy. Abstract, the 38 Leipziger Fortbildungsveranstaltung: "Labordiagnostik in der Bestandsbetreuung", Leipzig, Germany, 21st June 2013.
2. **El-Zahar Heba**, Müller Kerstin (2013): Determination of sodium and potassium levels in bovine blood by ion-selective electrodes and atomic emission spectroscopy. Poster, the 8th PhD-Symposium and DRS presentation seminar (Doktorandensymposium), 15th of July 2013.
3. **El-Zahar Heba**, Müller Kerstin (2013): Agreement between measurements of sodium and potassium in blood samples of cattle by ion selective electrodes and atomic emission spectroscopy. Poster, the DVG-Vet-Congress, Berlin, Germany 6-10 November 2013 Berlin.
4. **El-Zahar Heba**, Müller Kerstin (2014): Effects of Storage Conditions and Hemolysis on Sodium and Potassium Levels in Bovine Whole Blood Samples. Poster, the XIV Middle European Buiatrics Congress, Warsaw, Poland, 25-27 May 2014.
5. Müller Kerstin, **El-Zahar Heba** (2015): Fallstricke bei der Labordiagnostik von Störungen des Kaliumhaushaltes "Translated: Pitfalls in the laboratory diagnosis of disorders of potassium balance". Abstract, the 40 Jahre Präventivmedizin: "Zukunft gestalten – 40 Jahre Metabolic Monitoring", Leipzig, Germany, 19-20 June 2015.

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14 Selbständigkeitserklärung

Hiermit bestätige ich, **Heba Ibrahiem El-Zahar**, die vorliegende Arbeit selbständig und nur auf Grundlage der angegebenen Hilfsmittel und Quellen verfasst zu haben.

Berlin, den 23.02.2015

Heba Ibrahiem El-Zahar