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Effects of Oral Supplementation of Potassium Chloride in Hypokalemic Dairy Cows by Use of a Bolus Formulation on Metabolism, Abomasal Position and Vaginal Discharge Characteristics

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List of abbreviations

%	percent
°C	degree Celsius
1,25 OH vit D3	1,25 dihydroxyvitamin D3
AAS	atomic absorption spectroscopy
acetyl-CoA	acetyl coenzyme A
ANOVA	analysis of variance
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AV	abomasal volvulus
BE	base excess
BHBA	beta-hydroxybutyric acid
BID	bis in die (twice daily)
BUN	blood urea nitrogen
BW	body weight
CAD	cation-anion difference
DA	displaced abomasum
DCAD	dietary cation-anion difference
diff.	difference
DIM	days in milk
dist	distance between the caudal aspect of the xiphoid process and the caudal abomasal margin
DNA	deoxyribonucleic acid
e.g.	exempli gratia
ECF	extracellular fluid
EDTA	ethylenediaminetetraacetic acid
et al.	et alii/et aliae
etc.	et cetera
FAAS	flame atomic absorption spectroscopy
Fig.	figure
G/l	Giga per liter
Gamma-GT	gamma-glutamyltransferase
HCT	hematocrit
hrs	hours
ICF	intracellular fluid
ISE	ion-selective electrode
IU	international units
K_i	potassium concentration inside of the cell
K_o	potassium concentration outside of the cell
LDA	left displaced abomasum
M.	musculus
max.	maximum
min.	minimum
Mm.	musculi
mU/l	milliunits per liter
mus	muscle
N	number of units
NEFA	non-esterified fatty acids
p	probability value

PAS	Periodic acid-Schiff stain
pCO_2	carbon dioxide partial pressure
PLT	platelet count
prox	distance between the caudal aspect of the xiphoid process and the cranial abomasal margin
PTH	parathyroid hormone
r	Pearson's correlation coefficient
RAAS	renin-angiotensin-aldosterone-system
RBC	red blood cell count
ri-caud/le-caud	maximal right and left lateral extensions at the midpoint of the caudal half of the abomasum
ri-cran/le-cran	maximal right and left lateral extensions at the midpoint of the cranial half of the abomasum
RMP	resting membrane potential
SD	standard deviation
Sig.	significance
suppl.	supplementation
T/l	Tera per liter
TAG	triacylglycerol
WBC	white blood cell count
WW	wet weight

1 INTRODUCTION

Hypokalemia (moderate for serum potassium values < 3.5 mmol/l, severe for serum potassium values < 2.8 mmol/l) is frequently observed in high yielding dairy cows in the transition period (CONSTABLE, 2016). This time span, which is characterized by substantial physiological, nutritional and metabolic changes, ranges from two to three weeks pre partum until two to three weeks postpartum (BLOCK, 2010). Due to the abrupt drainage of potassium via milk, early lactation forms the highest risk for hypokalemia to occur. Potassium (K^+), a ubiquitous macromineral, is essential for a great number of body functions, among these acid-base homeostasis and transduction of neuro-muscular excitation. In cattle and other mammals, serum potassium concentration is maintained in a narrow range and reference values in cattle reported by different researchers range from 3.5 to 5.8 mmol/l (GOFF, 2004; CONSTABLE, 2016). Due to a generally high dietary K^+ load, mechanisms of K^+ excretion are well developed in ruminants. Therefore, whenever a (sudden) decrease in feed intake occurs, affected animals miss potassium sparing mechanisms and for this reason are at risk of developing hypokalemia.

The detection of disturbances in potassium homeostasis presents a challenge in the veterinary practice, as blood analysis is usually restricted to veterinary clinics and only sporadically performed by practitioners working in the field. The administration of potassium to animals at risk of developing hypokalemia via orogastric tube proves to be inconvenient under field conditions, however, the administration of medications to cattle in the form of solid boluses, which are administered by use of a balling gun, is well established. When applied correctly, the use of a bolus would provide an easy way to administer definite and thus safe amounts of potassium, also in absence of a precedent blood analysis.

The aim of the present study was to investigate the practical feasibility and efficacy of the administration of a bolus containing potassium and magnesium to hypokalemic dairy cows. Evaluation of the effects of the oral bolus formulation on the convalescence of the patient population considered the return of plasma potassium levels to normal as well as the improvement of the general condition considering the following aspects: return of appetite, an increase in the number of ruminal movements, an increase in milk yield, the stabilization of energy metabolism as represented by a decrease in the blood levels of non-esterified fatty acids and ß-hydroxybutyrate as well as the return of the blood glucose levels to normal and the effects on sodium, magnesium, calcium levels and acid-base balance.

2 LITERATURE REVIEW

2.1 Potassium

Potassium (K^+) is a macromineral with the atomic number 19. In the periodic table it is positioned in group 1, along with lithium, sodium, rubidium, caesium and francium. Elements in group 1 (alkali metals) have a single valence electron in their outer shell, which makes them highly reactive and likely to lose their valence electron to form cations with the charge +1. K^+ is cofactor of enzymes of protein and DNA synthesis. ATP-sensitive K^+ channels (K_{ATP}) are present in cardiac muscles and pancreatic beta cells, as well as in a wide variety of tissues, including skeletal muscle, brain neurons, peripheral axons and epithelial cells (ASHCROFT and GRIBBLE, 1999). Intracellular K^+ neutralizes fixed anions and is involved in the maintenance of acid-base homeostasis and cell volume (UNWIN et al., 2011).

Potassium is the most important intracellular cation and the third most abundant cation in ruminant tissues (SWEENEY, 1999). Only 2% of total body potassium is found in the extracellular fluid (ECF) compartment whereas the remaining 98% are mainly located intracellularly (ICF). Muscle cells form the greatest reservoir for potassium and for this reason play an important role in body homeostasis of potassium (BROBST, 1986).

As the resting membrane potential (RMP) is mainly a function of the ratio between intracellular and extracellular potassium (K^+) and sodium (Na^+) ions, changes in the K^+/Na^+ ratio result in alterations of the resting membrane potential of skeletal muscle cells. Focusing on potassium equilibrium, the diffusion potential of K^+ may be calculated using the Nernst equation (at 37 °C):

$$\text{Membrane potential (mV)} = - 61.5 \times \log([K_i]/[K_o]),$$

where K_i is the intracellular potassium concentration and K_o the potassium concentration outside the cell. Assuming that under normal conditions K_i = 150 mmol/l and K_o = 4 mmol/l, the concentration ratio between intracellular and extracellular space is 38:1 and the predicted membrane potential is -97 mV. Disregarding the cell's permeability for other ions, a small decline of the extracellular potassium concentration to 2.0 mmol/l, for instance, results in an increase of the RMP to -150 mV ($[K_i]/[K_o]$ = 75:1), making the cell much less excitable. A rise in extracellular K^+, on the contrary, decreases the K_i/K_o ratio and leads to a RMP closer to the threshold for opening Na^+ channels to initiate an action potential (GOFF, 2006). Myocardial cells react differently from skeletal muscle cells to alterations in K^+ concentrations. In the heart, hypokalemia has been shown to reduce K^+ channel conductance by inducing inactivation or delayed reactivation kinetics of voltage-dependent K-channels, thereby reducing outward repolarizing current and thus leading to a reduction in repolarization reserve. Furthermore, inhibitory effects of hypokalemia on the Na^+-K^+-ATPase in heart, as well as indirect effects of hypokalemia leading to activation of late Na^+ and Ca^{2+} currents, have been hypothesized to play a key role (WEISS et al., 2017).

The pH of an aqueous solution is defined as the negative decadal logarithm of the molar concentration of hydrogen (H^+) ions. A solution with pH 7 is described as neutral and blood pH of healthy cattle ranges between approximately 7.36 and 7.44. Changes in the acid-base-status affect the distribution of K^+. A decrease in extracellular pH is attributable to an increase in extracellular H^+ concentration, leading to a cellular uptake of H^+ and a shift of K^+ from the intracellular to the extracellular fluid compartment.

ARONSON and GIEBISCH (2011) explain the apparent K^+-H^+ exchange as the result of three possible regulatory pathways using the example of skeletal muscle cells. (1) Na^+ enters cells by Na^+-H^+ exchange and is extruded by the energy-dependent Na^+/K^+-ATPase. In the case of a fall in extracellular pH the rise in extracellular H^+ ions inhibits the Na^+-H^+ exchange, which leads to the accumulation of intracellular H^+ and a decline in intracellular Na^+ which in turn diminishes the activity of the Na^+/K^+-ATPase causing a decreased cellular uptake of K^+ and, thus, a rise in extracellular (interstitial) potassium concentration. (2) Na^+-bicarbonate (HCO_3^-) cotransport operates in parallel with the Na^+/K^+-ATPase. In case of acidosis with acidemia, the fall in extracellular HCO_3^- results in an accumulation of extracellular Na^+, inhibiting the Na^+/K^+-ATPase activity. That would cause a net loss of intracellular K^+. (3) Given, that there is a bicarbonate-chloride (Cl^-) exchange mechanism that works in parallel with a K^+-Cl^- cotransport, the fall of extracellular HCO_3^- in metabolic acidosis with acidemia would increase the inward movement of Cl^-. The resulting rise in intracellular Cl^- would then promote the efflux of K^+ via K^+-Cl^- cotransport. The result would again be the increase of the extracellular K^+ concentration. The latter deliberations suggest, that conversely, a rise in extracellular K^+ could cause a decrease in extracellular H^+ concentration (alkalinisation), whereas a decrease in extracellular K^+ would lead to acidification of the extracellular fluid.

As described above, in metabolic acidosis caused by inorganic anions (mineral acidosis), the decrease in extracellular pH will decrease the rate of Na^+/K^+-exchange and inhibit the inward rate of Na^+/HCO_3^- cotransport (PALMER, 2015). The resultant decrease in intracellular Na^+ will reduce Na^+/K^+-ATPase activity, causing a net loss of intracellular K^+. In addition, the decrease in extracellular HCO_3^- concentration will increase inward movement of Cl^- by Cl^--HCO_3^- exchange, further enhancing K^+ efflux by K^+- Cl^- cotransport. Loss of K^+ from the cell, however, is much smaller in magnitude in organic acidosis as for example in the setting of ketoacidosis. In this case, there is a strong inward flux of the organic anion and H^+ through the monocarboxylate transporters 1 and 4. Accumulation of the acid results in a larger fall in intracellular pH, thereby stimulating inward Na^+ movement by way of Na^+-H^{+-} exchange and Na^+-HCO_3^- cotransport. Accumulation of intracellular Na^+ maintains Na^+-K^+-ATPase activity, thereby minimizing any change in extracellular K^+ concentration (PALMER, 2015).

Potassium also plays an important role in the concept described as dietary cation-anion difference (DCAD). Numerous effects of increasing or decreasing DCAD of precalving rations have been shown, e.g. decreased sensitivity to parathyroid hormone in cows fed a strongly positive DCAD precalving diet, enhanced renal production of 1,25(OH) vitamin D3 in response to a low DCAD in the precalving diet, or increased responsiveness of target tissues to 1,25(OH) vitamin D3 associated with increased calcium absorption from the intestinal tract (LEAN et al., 2013).

Meta analyses on milk fever have shown that the risk of milk fever can be predicted from dietary levels of calcium, magnesium, phosphorus and dietary cation-anion balance (DCAD)(LEAN et al., 2013). The concept of dietary cation-anion difference (DCAD) of milk fever has its basis in the strong ion model of acid base balance as expressed in the (simplified) equation: $(Na^+ + K^+) – (Cl^- + S^{2-})$ (BLOCK, 1994). ENDER et al. (1962) showed, that dry cow diets low in potassium and sodium but high in chloride and sulfide or containing inorganic salts of other sources (low DCAD) when fed in the last two weeks before calving were able to prevent milk fever postpartum and cows fed an anionic diet starting six weeks before expected parturition in a study conducted by GAYNOR et al. (1989) tended to present with higher plasma calcium concentrations postpartum and lower incidence of milk fever when compared to cows fed more cationic diets.

It has been shown in sheep, that following an increase in dietary potassium rumen pH rises above 6.5, with a marked decline in the availability of magnesium (Mg) for absorption from the rumen (DALLEY et al., 1997). Furthermore, *in vitro* studies of MARTENS and KÄSEBIETER (1983) on isolated sheep rumen cells showed a decrease in trans-epithelial magnesium transport following a rise in the potassium concentration of the buffer solution, possibly due to the indirect inhibitory effect of extracellular K^+ on the Na^+/K^+-ATPase. However, in said experiments, Mg transport through rumen cells has been shown to be dependent on the function of the Na^+/K^+-ATPase, which could also be related to the Na^+-dependent Mg transport across ruminal cells as shown by SCHWEIGEL et al. (2006).

Whereas a negative CAD in the pre partum diet has been shown to exert a positive influence on calcium homeostasis postpartum, a CAD optimal for lactation rations, with regard to dry matter intake and milk yield, was shown to range from + 25 to + 50 mmol/l (SANCHEZ and BEEDE, 1996).

HARRISON et al. (2011) refer to a number of studies that clearly demonstrate that early lactation dairy cows are at a high risk to experience a negative K^+ balance. Their research showed that cows that were less than 75 days in milk tended to excrete greater amounts of K^+ than the cows in the calibration dataset, which was composed of a combination of data from various total collection metabolism trials, although K^+ intakes were similar in both groups. Thus, greater excretion and loss of K^+ via milk, even more so with often reduced dietary intake associated with calving, may result in a negative K^+ balance. Aforementioned authors conclude that supplementation of potassium in early lactation dairy cows results in increased milk yield and milk fat contents. WEST et al. (1987) compared the effects of dietary sodium bicarbonate (1.5 %) to varying concentrations of potassium carbonate (1.25 % and 1.85 %, respectively) on rumen environment, milk yield, milk composition and acid-base balance in lactating dairy cows. In their study, buffered diets resulted in higher milk fat percentages than control diets. Since cows offered diets containing potassium carbonate performed similar to those offered sodium bicarbonate, and potassium carbonate additionally serves as K^+ supplement, the authors recommend the use of potassium carbonate as rumen buffer. Added dietary potassium carbonate decreased unsaturated and trans-fatty acids and increased stearic acid (C18:0) content in milk fat samples, suggesting that there is a rumen-based mechanism for the noted increase in milk fat production (HARRISON et al., 2012). The latter statement is supported by observations of JENKINS et al. (2010), who demonstrated *in vitro*, that potassium is involved in the process of bio-hydrogenation of fatty acids at rumen level. According to the data of JENKINS et al. (2010) the bio-hydrogenation of unsaturated fatty acids to C18:0 is shifted toward an alternative pathway in low K^+ diets.

SILANIKOVE et al. (1997) suggest that additional potassium supplied at the onset of lactation might have a positive effect on dry matter intake and milk yield of cows.

2.1.1 Potassium regulation

The electric potential across the plasma membrane of body cells is driven by a concentration gradient of charged ions. A typical resting membrane potential ranges from −40 to −70 mV, with a net negative charge on the cytosolic side of the membrane. The maintenance of said resting membrane potential depends on the outward diffusion of potassium ions along their concentration gradient (WARREN and PAYNE, 2015). Secondly, a negative voltage created by active transport of cations out of cells by the energy dependent Na^+/K^+-ATPase is keeping K^+ inside of body cells, a transport protein, which can be found on the plasma membrane of most cells (HALPERIN and KAMEL, 1998). K^+ excretion and Na^+ conservation are more efficient in ruminants than in man (ANDERSON and PICKERING, 1962), possibly due to the

generally large dietary intake of potassium. Elimination of potassium is necessary to protect the body from severe alkalosis, since K^+ ions are exchanged in the kidney for hydrogen (H^+) ions and vice versa (PICKERING, 1965; WARD, 1966).

Total body K^+ is a function of two variables: (1) external K^+ balance, which describes the difference between K^+ intake and K^+ excretion (urine, feces, milk) and (2) internal K^+ balance or the distribution of K^+ between ICF and ECF (BROBST, 1986; SWEENEY, 1999)(Fig. 1). The dietary potassium requirement of lactating cattle is approximately 0.8 % of dry matter intake (NATIONAL RESEARCH COUNCIL, 2001) with more than 85 % of dietary potassium being absorbed by healthy cattle (SWEENEY, 1999). Since most forage fed to cattle are much higher in potassium content than required, normal potassium balance is maintained by elimination of excessive potassium via urine (75 %), feces (13 %) and milk (12 %) (WARD, 1966; SWEENEY, 1999). External potassium balance is mainly upheld through renal regulation of urinary excretion of potassium. Potassium is freely filtered across the glomerulus and urine may contain 5-200 % of the filtered potassium load, which indicates that the distal nephron is capable of K^+ secretion or reabsorption (BROBST, 1986; MUTO, 2001).

RABINOWITZ et al. (1985) showed that intravenous infusion of potassium chloride (KCl) in sheep resulted in an almost immediate and linear increase in renal potassium excretion. Also, shortly after dietary consumption of potassium, mechanisms of potassium elimination become active possibly following a kaliuretic reflex in which K^+ sensors in the splanchnic vascular bed detect local changes in K^+ concentrations and signal the kidney to alter K^+ excretion (RABINOWITZ et al., 1988; GREENLEE et al. 2009). In experiments conducted on rats, LEE et al. (2007) provided evidence for a gut factor that triggered immediate renal excretion of dietary potassium by a feed-forward control mechanism. An intragastric potassium infusion given with a meal to unfed animals led to greater renal clearance of plasma potassium than did the same intragastric infusion without the meal or potassium given by systemic infusion.

Muscle cells are the main reservoir for K^+ in mammals. Decreased plasma K^+ concentration, e.g. following deprivation of dietary K^+, activates feedback mechanisms in order to restore extracellular K^+ concentration. Hypokalemia for instance causes decreased expression of skeletal muscle Na^+/K^+-ATPase α2 isoform, resulting in a net K^+ “leak” from ICF into ECF (GREENLEE et al., 2009). Studies in rats using a potassium clamp technique afforded insight into the role of skeletal muscle in regulating extracellular K^+ concentration (PALMER, 2015). With this technique, insulin is administered at a constant rate, and potassium is simultaneously infused at a rate designed to prevent any drop in plasma K^+ concentration. The amount of potassium administered is presumed to be equal to the amount of K^+ entering the intracellular space of skeletal muscle. In rats deprived of potassium for 10 days, the plasma K^+ concentration decreased from 4.2 to 2.9 mmol/l. Insulin mediated K^+ disappearance declined by more than 90 % compared with control values. This decrease in K^+ uptake was accompanied by a 50 % reduction in both the activity and expression of muscle Na^+/K^+-ATPase, suggesting that decreased pump activity might account for the decrease in insulin effect. This decrease in muscle K^+ uptake, under conditions of K^+ depletion, may limit excessive decreases in extracellular K^+ concentration that occur under conditions of insulin stimulation. Concurrently, reductions in pump expression and activity facilitate the ability of skeletal muscle to buffer declines in extracellular K^+ concentrations by donating some component of its intracellular stores (PALMER, 2015).

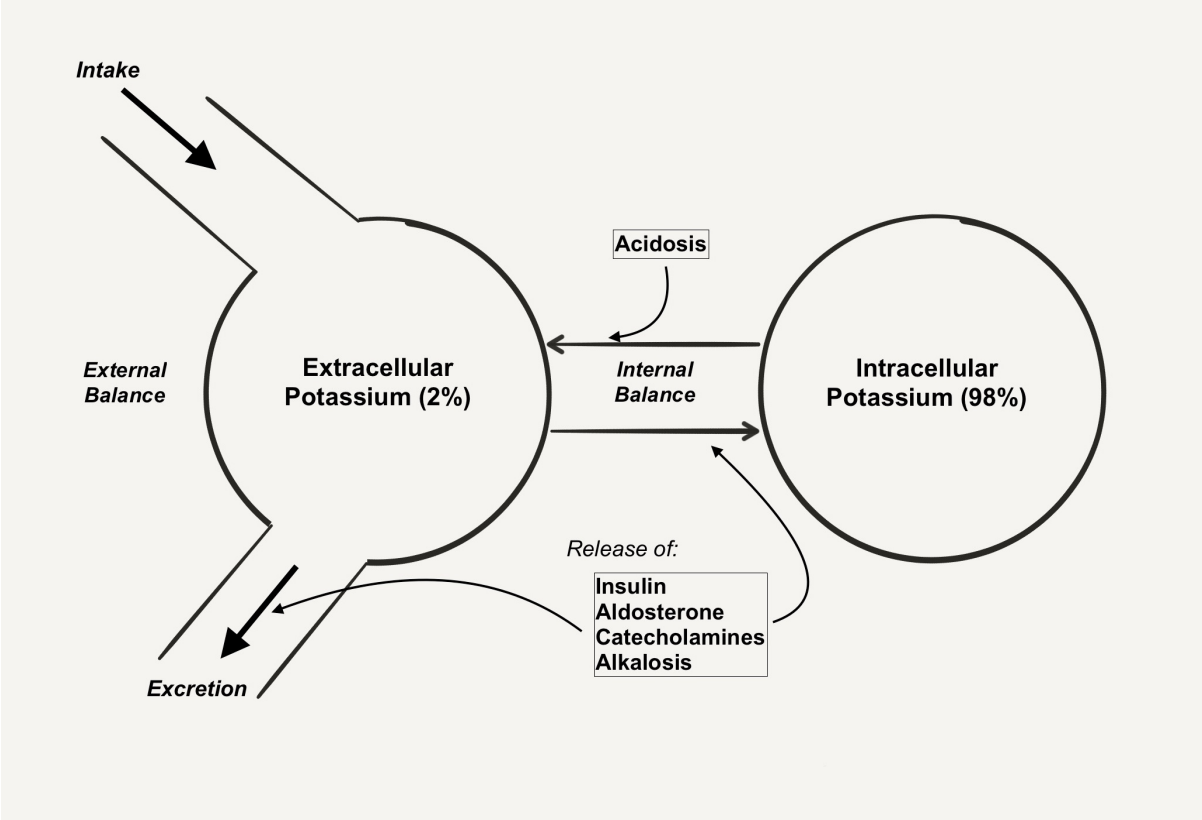

Fig. 1. Concentration of K^+ in extracellular fluid is determined by the external balance (intake versus excretion) and the internal balance (distribution of K^+ between extracellular and intracellular fluid compartments) (modified from BROBST, 1986).

An increased abundance of energy dependent H^+/K^+-pumps in renal collecting ducts drives active K^+ conservation in the kidneys and low plasma K^+ suppresses adrenal aldosterone release (MCDONOUGH et al., 2002, GREENLEE et al., 2009), as mineralocorticoids such as aldosterone have a plasma K^+ lowering effect. Aldosterone, which plays a major role in the regulation of blood pressure and fluid balance, is secreted by the adrenal cortex following the activation of the renin-angiotensin-aldosterone-system (RAAS). Aldosterone has been shown to influence both, internal and external potassium balance, by enhancing the uptake of K^+ into brain and body cells and by increasing the permeability of renal tubular membranes for K^+ and, thus, enhancing renal secretion of K^+ ions in exchange for Na^+ ions. Both mechanisms can lead to a decrease in plasma K^+ concentration (BROBST, 1986; RABINOWITZ, 1996; SIELMAN et al., 1997). High blood K^+ can directly stimulate the secretion of aldosterone, but low plasma Na^+ and low plasma volume are more potent triggers of aldosterone secretion. Therefore, hypokalemia may develop secondary to low plasma volume in dehydrated animals (GOFF, 2004). Treatment of dairy cows suffering from ketosis by use of corticosteroids with mineralocorticoid activity, e.g. isoflupredone acetate, has been associated with the occurrence of severe adverse effects caused by hypokalemia (SIELMAN et al., 1997; SATTLER et al., 1998; PEEK, 2003; COFFER et al., 2006).

Potassium is involved in the insulin-mediated uptake of glucose into liver and skeletal muscle cells (SWEENEY, 1999; CLAUSEN, 2003). Pancreatic insulin secretion following dietary intake of K^+ and glucose activates skeletal muscle and liver Na^+/K^+-ATPase, which pumps K from the ECF into these cells (GREENLEE et al., 2009; HO, 2011; PALMER, 2015). To this end, increased secretion rates of insulin subsequently to a meal prevent an excessive rise in

blood glucose and potassium concentrations. Intravenous administration of glucose or application of agents that enhance gluconeogenesis, which are commonly used in the treatment of ketosis in dairy cows, have been shown to lower the extracellular potassium concentration by stimulating insulin secretion (RUFF, 1999; JOHNS et al., 2004; COFFER et al., 2006).

Homeostatic mechanisms prevent imbalances; therefore, in healthy individuals, wide variations in potassium intake produce only small changes in total body potassium (RABINOWITZ, 1989; YOUN and MCDONOUGH, 2016).

2.1.2 Disorders in potassium homeostasis in bovines

2.1.2.1 Hypokalemia

Although substantial amounts of potassium are drained via the milk following the start of lactation, healthy periparturient cows have been demonstrated to be in a slightly positive potassium balance of approximately plus 60 g K^+, that is, if dietary intake is undisturbed (GOFF, 2006). Excessive potassium is excreted via a highly efficient mechanism by the kidneys. A deficient quality of certain components in the ration (usually silage) or diseases such as milk fever, ketosis, abomasal displacement (DA) or claw disorders can lead to a decrease in feed intake or even turn the cow to an anorectic status. When mechanisms of potassium excretion, e.g. via milk and urine are still ongoing and serum potassium cannot be replenished from the intracellular potassium stores in a sufficient rate, hypokalemia develops (SATTLER et al., 1998; SWEENEY, 1999; PEEK et al., 2003; HARRISON et al., 2011). In addition, osmotic diuresis as observed in humans excreting ketone bodies via the urine could also contribute to renal potassium losses in dairy cows with ketosis.

RABINOWITZ et al. (1988) demonstrated that experimentally induced starvation resulted in mild hypokalemia in sheep but failed to induce severe hypokalemia as observed in lactating dairy cows. In a study conducted by PRADHAN and HEMKEN (1968), lactating dairy cows that were fed potassium deficient rations (0.06 % and 0.15 % K^+ in dry matter) developed symptoms of potassium deficiency. Affected animals showed partial to complete inanition, pica as characterized by hair licking of stall-mates, floor licking and chewing of wooden partitions, as well as loss of glossiness of the coat within three to four weeks. Cows fed potassium deficient rations consumed about 34 % less than cows fed a ration adequate in potassium. There was furthermore a consistent decrease in milk potassium concentration during the period when cows were fed potassium deficient rations, however, a relatively greater decrease of potassium concentration in milk over that in blood plasma was observed. The authors conclude, that maintenance of the potassium reserve in the body in times of lower dietary potassium intake may be accomplished by reduced elimination of potassium through milk.

As mentioned above, potassium plays a major role in upholding the RMP. A decline in the extracellular K^+ concentration has been shown to raise the threshold potential of a stimulus necessary to induce muscle contraction. GOFF (2006) postulates, that the described hyperpolarization of the resting cell membrane due to hypokalemia reduces the amount of the neurotransmitter acetylcholine released by the nerve ending at the neuromuscular junction, which impairs the number of contracting muscle fibers.

In *in vitro* experiments, TÜRCK and LEONHARD-MAREK (2010) showed that a low K^+ concentration in the buffer solution reduces the activity of the circular muscle of the

abomasal corpus and, by this means, is able to affect the contraction activity of abomasal muscles. The latter mechanism could be of importance in the pathogenesis of abomasal displacement (DA). However, various disorders of the abomasum have been shown to result in hypokalemia themselves, caused by disturbances of the transport of gastric contents from the abomasum to the intestines (abomasal outflow disorders): posterior functional stenosis, abomasal volvulus, abomasal impaction, pyloric obstruction and functional stenosis of the ansa sigmoidea duodeni. While the passage of digesta to the intestines is hampered, a steady state secretion of hydrogen chloride (HCl) and consequently water into the abomasal lumen is continued, which subsequently causes the reflux of gastric contents from the abomasum into the rumen, a phenomenon which resembles vomiting in monogastric species. The fluid, however, is not lost in ruminants, but is sequestered in the rumen. The latter process results in hypokalemic, hypochloremic metabolic alkalosis (KUIPER and BREUKINK, 1980; DIRKSEN, 2006 b; CONSTABLE et al., 2013). Alkalosis causes a shift of potassium to the intracellular compartment via stimulation of a Na^+-H^+-exchanger and a subsequent activation of the Na^+/K^+-ATPase, leading to mild or severe hypokalemia (HALPERIN and KAMEL, 1998; KALAITZAKIS et al, 2010). Furthermore, hampered potassium absorption from the rumen following obstructive disorders of the gastrointestinal tract and renal compensation of metabolic alkalosis by excretion of bicarbonate, sodium, potassium and water via the urine, contribute to hypokalemia (KUIPER and BREUKINK, 1980). In cows fitted with a rumen and duodenal fistula the latter authors showed that - in case of obstruction of the proximal duodenum - abomasal reflux caused hypovolemia and hypokalemic, hypochloremic metabolic alkalosis while potassium and chloride levels in the rumen fluid increased. The latter authors postulated a hampered potassium absorption from the rumen due to high osmolarity of the rumen fluid and due to reduced rumen perfusion caused by hypovolemia.

In a study by KALAITZAKIS et al. (2010), cows with LDA had significantly lower median serum K^+ concentrations compared with control animals and the median K^+ concentration tended to decrease when fatty liver became more severe. The lowest potassium concentrations were measured in cows with a fatal outcome suffering from LDA and severe fatty liver syndrome. ROHN et al. (2004) compared clinical and laboratory parameters in cows that underwent surgery to correct LDA. Results showed that animals that were culled or slaughtered had lower serum potassium concentrations than those that were cured. In addition, hypokalemia was reported for cows with renal disorders and diarrhea as well as cows with ketosis, especially when the latter had been treated with mineralocorticoids (SIELMAN et al., 1997; SATTLER et al., 1998; PEEK et al., 2003).

2.1.2.1.1 Clinical findings in hypokalemia

In human medicine, a decreased potassium concentration is described as a common biochemical finding in cardiac patients, probably as an adverse effect of diuretic therapy or as a result of the endogenous activation of renin-angiotensin system and a high adrenergic tone. Hypokalemia is identified as an independent risk factor contributing to reduced survival of cardiac patients. In addition, hypokalemia is going along with increased incidence of sudden cardiac death caused by arrhythmia. Hypokalemia-induced arrhythmogenicity is attributed to prolonged ventricular repolarization, slowed conduction, and abnormal pacemaker activity (OSADCHII, 2009).

As a consequence of hypokalemia, dairy cows reveal flaccid paralysis of striated and smooth muscles. Fasciculation of skeletal muscles, typically of the anconeus muscle group due to muscle weakness, reflect the effects of hypokalemia on the resting membrane potential as described earlier. Profound muscle weakness of the lateral cervical muscles may force

affected animals to lie with their head resting in their flank, as the ligamentum nuchae, now supporting the majority of the head's weight, forces the poll to the side (JOHNS et al., 2004). 10 out of 14 hypokalemic cows in a retrospective study by SATTLER (1998) showed an abnormal position of head and neck, severe weakness, rumen hypomotility or -atony, abnormal feces, anorexia and tachycardia. Eight animals in the aforementioned study were suffering from recumbency with flaccid paralysis and a paretic gait when able to walk. Tachycardia was observed in eleven cows. In 17 cases of severe hypokalemia (K^+ < 2.1 mmol/l) cows showed profound muscle weakness and recumbency (PEEK, 2003).

COX (1982) produced experimental downer cows by fixating healthy cows in sternal recumbency with the right pelvic limb positioned under the body for 6, 9 and 12 hours, respectively. Prolonged recumbency may lead to terminal recumbency due to the crush compartment syndrome. Compression of limbs especially on hard flooring leads to direct muscle trauma and muscle ischemia and the compression of venous blood vessels causes edema of the affected limb, leading to a rising compartment pressure, which in turn exacerbates the ischemia and finally provokes neural injury and muscle infarction (MUBARAK and OWEN, 1975). Necrosis and myofiber vacuolation as well as necrosis of type-2 muscle fibers have been documented as signs of hypokalemic myopathy (SWEENEY, 1999; PEEK et al., 2000). In contrast to the response of the skeletal muscle, hyperpolarization of cardiac muscle cells increases excitability and delays repolarization, causing dysrhythmias (UNWIN et al., 2011). The effects of alterations in potassium homeostasis on cardiac functions have been associated with cardiac arrhythmias (FOSHA-DOLEZAL and FEDDE, 1988) and atrial fibrillation was diagnosed in 4 of 10, 2 of 14, 5 of 17, 1 of 7 and 2 of 15 cows with hypokalemia (SIELMAN et al., 1997; SATTLER et al., 1998; PEEK et al., 2000, COFFER et al., 2006; CONSTABLE et al., 2014).

2.1.2.2 Hyperkalemia

If renal function is normal and sufficient drinking water is available, cattle are well adapted to eliminate excessive potassium. Gross miscalculation or immoderate intravenous infusion of potassium chloride could result in iatrogenic hyperkalemia (SWEENEY, 1999). When fighting acidosis, the body reacts to rising extracellular H^+ load with the translocation of H^+ to the intracellular space and the shift of K^+ from intracellular to extracellular pools as described earlier. Therefore, hyperkalemia is a common complication in neonatal dairy calves suffering from acidosis due to diarrhea and can lead to life-threatening bradyarrhythmias, as well as skeletal muscle weakness (GRÜNBERG et al., 2011; SWEENEY, 1999). It is usually treated by infusion of glucose, insulin and sodium bicarbonate solutions (GRÜNBERG et al., 2011).

2.1.3 Analysis of potassium status

Potassium is the third most abundant mineral in ruminant tissues and most of it is located in the intracellular fluid of skeletal muscles. The terms hypo- and hyperkalemia, however, usually refer to the concentration of the agent in the blood and – more specifically – in the extracellular fluid compartment as represented by plasma or serum.

2.1.3.1 Analysis of plasma/serum samples

The determination of the concentrations of alkali metal ions in plasma or serum samples is often performed by use of ion-selective electrodes (ISE). These electrodes are fitted with membranes, which selectively take up ions from the sample solution, acquiring a charge and consequently a potential dependent on the activity of the ion in the solution. The activity is

related to the concentration of the ion. HAUSER (2016) names the ISE as the most attractive option to determine just one of the alkali metals because of their simplicity and low cost, which makes them suitable for field analysis. However, according to aforementioned author, the selectivity might be affected by the interference from other ions present in the sample.

Another common method for quantification of a great number of elements is the atomic absorption spectroscopy (AAS). The elements in the sample are atomized and vaporized, for example by use of an acetylene flame (flame atomic absorption spectroscopy = FAAS), ending up as free and neutral atoms. A light source emits light of a given wavelength, which, passing through the atoms of the sample, is absorbed partially. The intensity of the light behind the atomizer is measured in a detector and compared to light passing through the unit without a sample. The ratio between both values is interpreted using the Beer-Lambert law, which correlates the absorbance of light to the properties of the attenuating material.

Potassium values measured in serum samples are often slightly higher than plasma potassium concentrations because of possible delays in sample processing and/or the effect of clotting, which causes depletion of potassium ions from platelets (NIJSTEN, 1991; UNWIN et al., 2011). Delays in sample processing can cause pseudohyperkalemia even before hemolysis is visible. In the latter case the erythrocyte's Na^+/K^+-pump does not operate properly due to shortage of glucose, which can lead to potassium leakage out of the cell. SCHULZE (2009) showed that in non-centrifuged full blood samples stored at room temperature, potassium concentrations of more than 5 % of the samples had exceeded limit values after 4 hours. The author points out, however, that cooling of samples can also lead to a rise in extracellular K^+ concentration, as lower temperatures may hamper the function of the Na^+/K^+-ATPase. In a study conducted by NIINISTO et al. (2007), changes of potassium concentration in equine red blood cell concentrate, however, were only significant from 7 days of storage onwards at a mean storage temperature of 3.6 °C (range 2.4 – 3.8 °C).

2.1.3.2 Analysis of erythrocytes

Disturbances of acid base balance most commonly affect the shift of potassium into or out of body cells. Therefore, the potassium concentration in extracellular fluid does not necessarily reflect the status of total body stores of the cation (BROBST, 1986; ARONSON and GIEBISCH, 2011). JANOWITZ (1990) used blood plasma and hemolyzed whole blood samples to determine and evaluate the intra-erythrocytic electrolyte concentrations of cows suffering from DA. The intra-erythrocytic concentrations of sodium, potassium and chloride were calculated using the equation:

$$Ke\ (mmol/l)\ = \frac{Kh - Kp\ (100 - Hkt\ \%)}{Hkt\ \%}$$

(K_e = intracellular electrolyte concentration; K_h = hemolysate electrolyte concentration; K_p = plasma electrolyte concentration). The findings in the latter study showed a wide range of intraerythrocytic concentrations of analyzed electrolytes in healthy cows, from which the author concluded, that intracellular changes in electrolyte homeostasis cannot be interpreted by the examination of a single sample. In a study conducted by CARLMARK et al. (1982), no statistically significant correlations were found between potassium values of erythrocytes and potassium concentrations measured in muscle biopsy samples. On the contrary, SCHNEIDER et al. (2016) showed, that K^+ values differed significantly between erythrocytes and muscle tissue. By inheritance cattle belong either to those carrying erythrocytes with high,

intermediate or low potassium contents, respectively (CHRISTINAZ and SCHATZMANN, 1972).

2.1.3.3 Analysis of muscle tissue

Determination of potassium levels in muscle tissue obtained by biopsy allows to evaluate the interstitial and intracellular pool of potassium and to this end should help to estimate the total body potassium concentration. The use of muscle biopsy material for evaluation of potassium homeostasis is a routine procedure in sportsmen and has also been applied in cattle (VEENEKLAAS et al., 2002). The *Bergström* needle is used for routine semi-open muscle biopsies for diagnostic reasons and research (BERGSTRÖM, 1975). BOUWMAN et al. (2010) used a modified *Bergström* needle (Maastricht Instruments, Maastricht, The Netherlands) for obtaining samples from the *M. vastus lateralis* in standing horses following local anesthesia (lidocainhydrochlorid plus adrenalin) at a point located 15 cm ventral from the center of the *tuber coxae* and 7 cm caudal from the cranial border of the muscle at a depth of 5 cm. Other authors propose the *Mm. biceps femoris*, *semimembranosus*, or *semitendinosus*, respectively, as a sampling site in cattle (VEENEKLAAS et al., 2002).

2.1.4 Reconstitution of potassium homeostasis

OETZEL (2007) recommends the administration of potassium chloride at a dosage of 100 g to early lactating (days 5 to 28 postpartum) dairy cows at risk (anorexia, ketosis). Oral supplementation of K^+ in periparturient dairy cows with experimentally induced hypokalemia was shown to increase plasma and milk potassium levels while fat mobilization decreased following potassium supplementation (CONSTABLE et al., 2013). In another study, alterations in the spectrum of fatty acids in milk were observed following oral administration of potassium, which were related to potassium dependent changes in ruminal fermentation patterns (JENKINS et al., 2010). Potassium is thought to improve and regulate abomasal and uterine motility and to this end could contribute to a reduction in the incidence of abomasal displacement (DA) and to the enhancement of the uterine mechanism of self-cleansing (BRAINARD et al., 2007; TÜRCK and LEONHARD-MAREK, 2010).

In a case report of DAVIES et al. (2008), severe hypokalemia and kaliuresis in a dog suffering from hyperadrenocorticism led to paresis and firm muscles. Infusion of KCl at a rate of 0.2 mmol/kg/h led to correction of K^+ concentration and improvement of muscle function. Intravenous administration of solutions containing KCl to dairy cows in amounts that do not interfere with cardiac function, however, has been shown to fail with respect to reconstitution of the serum potassium levels (GOFF, 2006). An increased urine flow following intravenous infusion of KCl may furthermore even increase urinary potassium elimination (SWEENEY, 1999).

ANDERSON and PICKERING (1962) refer to a number of experiments concerning plasma clearance of potassium. In the latter study, plasma potassium concentrations of cows rose 1-2 milliequivalents/l as a result of the infusion of KCl, but a progressive increase in plasma potassium did not occur. Within two hours of the onset of infusion, the rate of elimination of potassium had risen to approximately equal the rate of administration and clearance measurements indicated an excess of potassium excreted over that filtered.

WARD (1966) report on experimental oral administration of more than 230 g potassium (approximately 450 g KCl) resulting in death, whereas smaller quantities did not. However, 500 g of KCl dissolved in 8 L of water given by orogastric tube in addition to intravenous

administration of fluids supplemented with KCl was able to increase potassium concentration to levels within the reference range within 6 hours but lead to diarrhea (SIELMAN et al., 1997).

Administration of potassium chloride via the oral route has, nevertheless, been demonstrated to form a safe way to reconstitute the potassium level in hypokalemic animals and humans within a short period of time (GREENLEE et al., 2009; CONSTABLE et al. 2014). In experiments conducted at the Ruminant and Swine Clinic, Freie Universität Berlin, the potassium balance was explored in healthy cows under steady state conditions and following oral administration of 40 g potassium chloride twice daily. It could be observed that plasma potassium levels increased within on average 60 minutes (OTT et al., 2010). SWEENEY (1999) suggests a free-choice oral electrolyte solution composed of 60 g sodium chloride (NaCl) and 30 g KCl dissolved in 15 L of water in cases of mild hypokalemia and, in more severe cases, administration of 120 up to 240 g KCl 2 to 3 times daily. GOFF (2006) recommends oral therapy with KCl at a dosage of 100-150 g/dose twice daily (BID) in several gallons of water. With hypokalemia being common in cows with LDA, RDA or abomasal volvulus (AV), KCl should routinely be administered orally to dairy cows in which hypokalemia can be suspected (CONSTABLE, 2013).

Since dehydration activates the RAAS and aldosterone promotes potassium elimination through the kidneys, plasma volume should be corrected first, if necessary (GOFF, 2006)

2.2 Abomasum

2.2.1 Anatomy

The abomasum is a pear shaped organ with a capacity of approximately 28 liters (BUDRAS and WÜNSCHE, 2002). It is located on the right side of the rumen and has contact to the ventral abdominal wall. Most of the corpus is located left of the ventral median and the pylorus extends to the right side of the cow caudal to the omasum (GOFF and HORST, 1997; DIRKSEN, 2006 a).

2.2.2 Ultrasonographic assessment

Size, position and contents of the abomasum can be assessed using a 3.5 to 5 MHz linear or convex ultrasound transducer (BRAUN, 2009). Due to its position adjacent to the abdominal wall and its heterogeneous and moving contents, the ultrasonographic visualization of the abomasum has been described as easy and practicable (BRAUN et al., 1997). Its cranial margin can be visualized approximately 8 cm caudal of the xyphoid process from the left and right paramedian regions and the ventral midline. Fig. 7 shows a scheme of the ultrasonographic measurements on a sagittal section from a dorsal view (BRAUN et al., 1997).

2.2.3 Abomasal motility

GEISHAUSER (1995) refers to numerous studies saying that the dilatation of the abomasum and an accumulation of gas are prerequisites for DA. There is always some gas in the abomasum, which collects in the dome of the fundus, the starting point for the displacement of the abomasum. By insufflation of air into the abomasum of cows with DA, various degrees of displacement could be produced, whereas insufflation of gas into the abomasum of healthy cows did not cause dilatation or displacement. Therefore, it was assumed, that a

disturbance of the emptying process of gas from the abomasum plays a role in the accumulation of gas and consequently the etiology of DA (DIRKSEN, 1971). According to DOLL et al. (2009), the decrease in abomasal motility is thought to be the key event in the majority of cases of abomasal displacement.

The role of potassium in the pathogenesis of abomasal displacement was investigated by *in vitro* experiments by TÜRCK and LEONHARD-MAREK (2010). In their experiments abomasal muscle cells of the corpus and pyloric region were placed in incubation chambers. An increase of potassium in the buffer solution, representing the extracellular fluid compartment, had a positive influence on the contraction activity of the incubated muscle cells as measured in millinewton (mN). Insulin on the other hand, within pathophysiological levels of 20 to 80 mU/L (reference levels range from 4 to 11 mU/L), had a direct inhibiting effect on the muscles of the abomasal corpus. The effect of potassium on smooth muscle cells was also described by MORGAN and SZURSZEWSKI (1980), who showed an increase in tension of smooth muscles isolated from the canine stomach when exposed to a higher K^+ concentration. HOLTENIUS et al. (2000) studied the effect of plasma glucose levels on the abomasal outflow rate in dairy cows using a hyperinsulinemic clamp technique. At euglycemia, induced hyperinsulinemia resulted in a significant reduction of abomasal fluid outflow *in vivo*. Similar findings were made by ELIASSON (1995), who showed reduced gastric and proximal intestinal motility in hyperinsulinemia at euglycemia in humans. The addition of potassium channel blockers and the reduction of Na^+/K^+-ATPase activity with the use of ouabain both antagonized the inhibiting effects of insulin on the muscle cell contraction in the study performed by TÜRCK and LEONHARD-MAREK (2010), which leads to the conclusion that the effects of insulin are related to its effect on the insulin-mediated cellular uptake of potassium.

2.2.4 Abomasal displacement (DA)

The left displacement of the abomasum (LDA) is characterized by the movement of the dilated abomasum between the rumen and the left abdominal wall and frequently occurs in adult, lactating dairy cows (CONSTABLE et al., 1992). The transition period, ranging from two to three weeks pre partum until two to three weeks postpartum, is the major risk period for the development of LDA and 80 – 90 % of affected animals are diagnosed within one month after parturition (SHAVER, 1997).

Abomasal hypomotility is considered an important factor in the etiopathogenesis of the abomasal displacement (DA), allowing accumulated gas to promote enlargement and dislocation of the organ (WITTEK et al., 2005). VAN WINDEN et al. (2004) managed to induce left displacement of the abomasum in dairy cows in an experimental setting by feeding a mixture of maize silage and concentrates, which indicates an association of DA with a high level of rapidly fermentable components in the ration fed postpartum. GOFF and HORST (1997) suggest a small roughage mat in the rumen as a risk factor for the condition. A high body condition and high plasma concentration of non-esterified fatty acids (NEFA) in the pre partum period are named as significant risk factors for DA (CAMERON et al., 1998) and hypocalcemia near the time of calving increases the risk of developing LDA (Massey et al. 1993). Furthermore, VAN WINDEN et al. (2003) suggest that fatty liver, elevated blood ketone concentrations and high activity of aspartate aminotransferase (AST) might play an important role in the pathogenesis of LDA.

2.2.4.1 Diagnosis of abomasal displacement

The dislocated abomasum can only be detected via rectal palpation if the dilated organ is enlarged enough to reach the pelvic cavity. In some cases, the tense caudal margin of the omentum majus or the rumen, which might be shifted to the right in cases of LDA, can be felt (DIRKSEN, 2006 a). A full paralumbar fossa can indicate a DA but is not pathognomonic either. The diagnosis can be secured performing percussion and auscultation (RICHMOND, 1964) although it should be kept in mind that any gas-filled tensioned organ can produce the metallic sound that is usually auscultated over a DA.

2.2.4.2 Correction of left displaced abomasum

Treatment of the left displacement of the abomasum includes non-invasive techniques and various surgical approaches. The aim of the different surgical approaches is to achieve a long-lasting fixation of the abomasum in its physiological position. This aim can be realized either by fixation of the omentum (omentopexy) or the organ itself (abomasopexy or pyloropexy) at an appropriate site on the body wall.

2.2.4.3 Conservative treatment

In first instance the cow is brought into right recumbency. Subsequently, the animal is brought in dorsal recumbency and rolled on its back until the dislocation is no longer detectible. The recurrence rate in treating LDA conservatively is high, especially if the condition was not detected at an early stage (DIRKSEN, 2006 a).

2.2.4.4 Surgical correction of left displaced abomasum

There are different surgical methods established in order to restore abomasal outflow and bring the abomasum back in its physiological position.

A longer-lasting fixation of the abomasum at its physiological position can be performed with the closed suturing technique (HULL, 1972). The cow is rolled into dorsal recumbency over its right side and the ventral abdominal wall is checked for the gas filled abomasum via percussion and auscultation. After insuring the proper positioning of the organ by auscultation, it is fixated with a curved needle and a synthetic thread (blind stitch) and the patient is rolled into left lateral recumbency. Complications reported for the closed suturing technique include: erroneous puncture of the mammary vein, provoking a torsion of the mesentery root, perforation of other abdominal organs, peritonitis, local infection and fistulation (DIRKSEN, 2006 a).

In order to insure the proper fixation of the abomasum, GRYMER and STERNER (1982) developed the “roll and toggle” procedure. A small trocar cannula is forced through the abdominal wall into the abomasum where a steelband effect has been detected after having brought the in the animal into dorsal recumbency. After removing the stylet from the cannula the gas and fluid leaking from the trocar opening can be checked for their distinct smell and low pH to ensure proper placement of the trocar inside the abomasum. A suture wire with a polypropylene bar (toggle pin) on one end is pushed through the trocar into the abomasum and the procedure is repeated 2-3 inches (4-7 cm) anterior to the first toggle suture. The two suture ends are then tied together over a gauze bandage (GRYMER and STERNER, 1982; DIRKSEN, 2006 a).

A technique often performed in field practice is the two-step laparoscopic repositioning and ventral fixation of the dislocated abomasum (JANOWITZ, 1998), which allows the surgeon to visually explore the abdominal cavity and introduce one toggle-bar under sight. As with the method described by GRYMER and STERNER (1982), the organ is fixated at the ventral abdominal wall. While the two-step laparoscopic abomasopexy requires to put the animal in dorsal and subsequently lateral recumbency, the one-step techniques do not require the latter step (BARISANI, 2004; CHRISTIANSEN, 2004; KREHER, 2008)

Other methods require laparotomy, which allows the surgeon to fully explore and evaluate the abdominal cavity and ensure proper identification and fixation of the abomasum itself or of the greater omentum. The “Utrecht” method is performed via left paralumbar laparotomy; the organ is degassed, replaced and the greater omentum is fixated to the ventral abdominal wall (LAGERWEIJ and NUMANS, 1962; DIRKSEN, 2006 a). In the “Hannover” technique, a right flank laparotomy is performed and the abomasum is located manually. The accumulated gas is released using a needle connected to a rubber tube. The abomasum can now be replaced into its correct position on the right hand side of the abdomen. The omentum adjacent to the sphincter region and the pylorus is brought into sight. The omentopexy is performed a hand’s width from the pyloric sphincter with the help of non-absorbable suture wire and two polyamide plates (DIRKSEN, 1967; SEXTON et al., 2007).

2.3 Further diseases of the transition cow

As described earlier, the transition period of the cow is characterized by dramatic metabolic, physiologic and nutritional changes. Parturition as well as the onset of lactation mark events of increased energy requirement. A decrease or complete absence of feed intake is a general feature around calving (RABOISSON et al., 2014), which may contribute to a negative potassium balance. Postpartum diseases like milk fever, trauma caused by dystocia, LDA, metritis, mastitis or ketosis can exacerbate negative energy balance.

2.3.1 Hepatic lipidosis

During periods of low feed intake, the body adapts to the reduced dietary intake of carbohydrates by synthesis of carbohydrates from proteins. To protect the body against consumption of too much body protein, adaptive mechanisms exist to direct the mobilization of reserve energy from fat. The mobilization of body fat and breakdown of triglycerides results in the release of NEFA from adipose tissue, which are available as fuels to most body tissues (HERDT, 2000). Complete oxidation of NEFA in the liver generates acetyl-CoA, which can be used to generate energy via the Krebs cycle. When postpartum mobilization of fat and release of NEFA exceeds the needs and the Krebs cycle is overloaded, acetyl-CoA is used for the production of ketone bodies and NEFA are re-esterified to triglycerides, which are stored in hepatocytes, leading to the development of hepatic lipidosis, also referred to as fatty liver (GRUMMER, 1993; ESPOSITO et al., 2014).

2.3.2 Acetonemia and acidosis

In the systemic circulation, ketone bodies can serve as an additional energy source for muscle (HERDT, 2000). An increased blood concentration of ketone bodies without clinical signs of ketosis is described as subclinical ketosis and has been defined as a concentration of blood serum beta-hydroxybutyric acid (BHBA) of > 1.2 mmol/l (MCART et al., 2011) or > 1.4 mmol/l while clinical symptoms have been related to serum BHBA concentrations exceeding 3.0 mmol/l (ANDERSSON, 1988; DUFFIELD et al., 2009). RABOISSON et al.

(2014) refer to a number of studies stating that increased concentrations of BHBA or NEFA are associated with an increased risk of developing various diseases and reproductive disorders.

In humans, type 1 diabetes mellitus may lead to diabetic ketoacidosis, an acute metabolic complication characterized by hyperglycemia, hyperketonemia and metabolic acidosis. In these patients, profound hypokalemia is usually a side effect of an intracellular potassium shift and increased urinary potassium loss following treatment with insulin; however, cases of hypokalemia in the setting of diabetic ketoacidosis prior to treatment have been reported. According to DAVIS et al. (2016), this may be due to osmotic diuresis, emesis and secondary hyperaldosteronism but occurs rarely, as acidosis and insulin deficiency usually lead to an extracellular shift of potassium. Noteworthy, insulin-dependent diabetes mellitus has been described in cattle, i.e. in context of metabolic disorders such as hepatic lipidosis (CIOBOTARU, 2013). HOLTENIUS and HOLTENIUS (1996) describe two types of metabolic settings in which ketosis may occur – the hypoglycemic-hypoinsulinemic type and the hyperglycemic-hyperinsulinemic type, whereas the latter has similarities with the initial stage of non-insulin dependent diabetes mellitus (type 2) in humans. In the setting of negative energy balance in early lactation, increased lipolysis, ketogenesis and utilization of ketone bodies serve as protein sparing mechanisms to protect the body from hazardous protein degradation. In these cows, plasma glucose and insulin levels are low and concentrations of free fatty acids and ketone bodies are high; however, this classical (primary) type of ketosis is generally not combined with other diseases, as the hyperketonemia may simply be seen as a consequence of change in metabolism. The other form of metabolic disorder associated with hyperketonemia as described by the authors is characterized by hyperglycemia and hyperinsulinemia. Animals affected of this so called secondary ketosis show signs of insulin resistance and glucose tolerance as well as varying degrees of fatty liver. In this case, an important predisposing factor is overfeeding in the dry period.

Cattle with abomasal outflow disorders usually show moderate to severe hypochloremic, hypokalemic alkalosis; however, extremely advanced abomasal volvulus may lead to metabolic acidosis due to devitalization of affected organs and subsequent lactic acidosis. Cows with a large abomasal volvulus, dehydration and weakness combined with metabolic acidosis have been shown to have a grave prognosis. By extension, if metabolic acidosis is accompanied by hypochloremia and hypokalemia in these animals, the possibility of severe ketoacidosis should be investigated as well (FUBINI and DIVERS, 2007).

2.3.3 Retained placenta and metritis

Retained placenta and uterine infection after parturition are manifestations of a reduced immunity in the periparturient period (MULLIGAN and DOHERTY, 2008). A case of puerperal metritis is defined as an animal with abnormally enlarged uterus, fetid, watery, red-brown uterine discharge, signs of systemic illness like decreased milk yield and signs of toxemia combined with fever > 39.5 °C within 21 days postpartum whereas animals with an abnormally enlarged uterus and a purulent uterine discharge detectible in the vagina within 21 days after parturition but without signs of systemic illness may be classified as having clinical metritis. 21 days or more postpartum, the presence of purulent or mucopurulent exudate in the vagina classifies clinical endometritis (SHELDON et al., 2006). Although lacking systemic signs, clinical endometritis can lead to significant economic losses due to decreased reproductive rate, decreased feed intake, reduced milk yield and increased culling rate (LEBLANC et al., 2002).

There are several diagnostic techniques for detection of endometritis in clinical practice, including transrectal palpation, vaginal inspection and inspection for vulvar discharge using a vaginal device (Metricheck™, Hamilton 3242, Simcro, New Zealand) (HEUWIESER et al., 2000; PLETICHA et al., 2009).

2.3.4 Mastitis

During the dry period, which begins averagely at month 7 of gestation, the mammary gland undergoes several changes. Involution of the udder includes apoptosis of mammary epithelium, which leads to an increased number of neutrophils and macrophages in the mammary gland. Within the first week of dry period, a keratin-like protein forms a plug in the streak canal to prevent bacterial entry to the udder and increased concentration of iron-binding lactoferrin prevents bacteria from growing. However, as milk flow ceases to flush bacteria from the milk canal, a high number of new intramammary infections may occur. These intramammary infections can result in clinical mastitis, which, overall, is the result of infection established during the dry period or early lactation and is most likely to occur during the first month of lactation (GOFF and HORST, 1997).

3 STUDY OBJECTIVE

The aim of the present trial was to evaluate the effects of administration of an oral bolus formulation containing potassium on the convalescence of hypokalemic dairy cows with plasma potassium levels below 3.5 mmol and periparturient disorders including abomasal displacement to the left (LDA), ketosis, metritis and mastitis as single entities or in combination. The reference range for plasma potassium of 3.5 – 5.0 mmol/l was explored for the laboratory of the Ruminant and Swine Clinic by the herd health service of the clinic in accordance with the settings of the instruments used in the laboratory of the clinic (EML 105 Analyzer, Radiometer Copenhagen, Bronshoj, Denmark). The evaluation considered the following criteria:

1. The return of the plasma potassium level within the reference range of 3.5 -5.0 mmol/l
2. The return of the appetite as expressed in an increase in the daily feed intake (in kg wet weight).
3. The increase in the number of ruminal movements as expressed in the number of ruminal contractions over a period of 3 minutes.
4. The increase in milk yield within the observation period (5 calendar days).
5. The position of the abomasum as evaluated by ultrasonographic examination.
6. The stabilization of the energy metabolism as represented by a decrease in the blood levels of non-esterified fatty acids and ß-hydroxybutyrate and the return of the blood glucose levels to normal.
7. The effects of potassium supplementation on sodium and calcium levels as well as the acid-base balance.

4 MATERIALS AND METHODS

4.1 Study Design

This randomized controlled clinical trial was conducted as a phase 1/phase 2 study (safety/efficacy) at the Ruminant and Swine Clinic, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany (in the following referred to as “the clinic”) in the time span between August 2012 and June 2013. Animals entered the study, when the results of the clinical examination at admission and the laboratory analysis performed at the same time demonstrated that the inclusion criteria as given in 4.2.3 were met.

Documentation of data was performed by written protocols, electronic data sheets (Microsoft Excel 2010) and the veterinary practice management software program (EasyVET, IFS GmbH, 30177 Hannover, Germany) used by the clinic. Study animals were examined and treated by the doctoral student under supervision of the research investigator. Trained animal care attendants were responsible for feeding, milking and cleaning of the stable. The study was performed in agreement with the requirements of the committee for animal experimentation and is authorized by local authorities (LAGeSo Turmstraße 21, 10559 Berlin, Germany) under the registration number G 0376/12.

4.2 Animal Selection and Identification

4.2.1 Details of Study Animals

Lactating dairy cows that were referred to the clinic in the time span between August 2012 and June 2013 by local practitioners were allotted at random using a randomization scheme (Microsoft Excel for Mac 2011, Version 14.0.0) either to the treatment group (Group 1a) or the control group (Group 1b), when these demonstrated moderate hypokalemia with plasma potassium levels of ≥ 2.8 and < 3.5 mmol/l. All animals displaying severe hypokalemia with plasma potassium levels of < 2.8 mmol/l were allotted to Group 2 and received a treatment as for ethical reasons withholding treatment was not feasible due to complications inherent with severe hypokalemia as described earlier (SATTLER et al., 1998; PEEK et al., 2000).

4.2.2 Disease History

A questionnaire concerning the patient history of four weeks preceding the application of the bolus and following the return of the cow to its farm of origin was sent to the herd managers, who were asked to fill in the document on basis of data obtained from the herd management program or from treatment records. The information included: the patient history beginning four weeks before application of the bolus as well as information on the period following dismissal from the clinic. The latter information considered, whether the animal was still in the herd, its milk yield, the days to first service and to pregnancy, respectively.

4.2.3 Inclusion Criteria

Lactating dairy cows with confirmed hypokalemia (plasma potassium levels as determined by ion-selective electrode (ISE) < 3.5 mmol/l) at admission suffering from the following diseases were eligible:

Left displacement of abomasum (LDA), (sub-) clinical ketosis (the blood ß-hydroxybutyrate level being > 1.2 mmol/l as determined by Accucheck Aviva® instrument; Roche Diagnostics, Basel, Switzerland), metritis, mastitis, either alone or in combination.

4.2.4 Exclusion Criteria

Recumbency, fractures, purulent arthritis, diffuse peritonitis, right displacement of abomasum, severe disturbance of the general condition that required a "rescue treatment", which includes intravenous administration of large volumes of fluids (exceeding 10 L normal (0.9%) saline), the administration of dexamethasone, relaparotomy or other invasive procedures, the change of the antibacterials from cephalosporines to another group of antibacterials.

4.2.5 Animal Management and Housing

Animals were kept in separate units representing free stalls with straw bedding at the clinic and were fed twice daily at 7 o'clock a.m. and 2 o'clock p.m.

4.3 Treatments

4.3.1 Administration of Investigational Product(s)

The product used in the present study was a potassium containing preparation having the following composition as described by HOEJVANG-NIELSEN (2016, EP 2887820 B1):

- Potassium chloride: 51 wt.-%
- $CaCl_2$ $2H_2O$: 25 wt.-%
- Water: 15 wt.-%
- Magnesium oxide: 9 wt.-%

The bolus formulation contained 45 to 48 g potassium per piece, which is equivalent to approximately 85 to 91 g potassium chloride. High ruminal K^+ concentrations decrease the apparent digestibility of magnesium in ruminants because of impaired magnesium absorption from the forestomachs (SCHWEIGEL AND MARTENS, 2000). Therefore, approximately 15 to 16 g magnesium oxide was added in order to prevent iatrogenic hypomagnesemia in treated cows.

Boluses were applied by the oral route using a bolus gun. For the animals in the treatment groups (1a; 2) the bolus was placed in the bolus applicator with the rounded end pointing forward. The applicator was introduced into the oral cavity of the cow such that the tip of the applicator was sited just cranial to the opening of the esophagus or it was located in the proximal part of the esophagus. The handle was pressed to release the bolus, which was subsequently swallowed by the animal or passed through the esophagus lumen into the rumen. The applicator was removed immediately from the oral cavity.

Cows in the non-treatment control group (Group 1b) received a sham treatment by introducing the empty balling gun into the oral cavity.

4.3.2 Summary of Use, Disposal or Return of Boluses

A total of 50 boluses were administered to cows assigned to the treatment groups as displayed in Table 1. Two boluses broke before administration, and two boluses had to be discarded due to mishandling by the administrator. A total of 234 boluses were returned to the producer.

Table 1. Overview of boluses used for treatment of hypokalemic cows.

	Group			
	1a	1b	2	All
Number of boluses				
1	13	1*	0	14
2	6	0	9	15
3	0	0	2	2

* Cow received rescue treatment on day 4

4.4 Study Procedures

4.4.1 Sampling Procedures

Blood samples were drawn from the jugular vein into appropriate tubes fitted either with lithium-heparinate or potassium EDTA (Sarstedt AG & Co. KG, 51588 Nümbrecht, Germany)(s. 4.4.2.1).

Urine was collected by manual stimulation of the escutcheon area ventral of the vulva.

Suction assisted muscle biopsy with a modified *Bergström* needle (Walter Veterinär-instrumente eK, 15837 Baruth, Germany), was performed to obtain samples from the vastus lateralis muscle (LEITNER, 2009). An area of approximately 15 cm x 15 cm was shaved and cleaned and 10 ml Isocain 2% (procainhydrochloride plus epinephrine, Selectavet, Dr. Otto Fischer GmbH, 83629 Weyarn-Holzolling, Germany) were injected subcutaneously. Following a small incision of skin and fascia of 1-1.5 cm length, two biopsy specimens were obtained at a depth of approximately 5 cm. In order to retrieve samples viable for analysis of intracellular components it was important to retrieve intact muscle tissue. Since the original technique as described by BERGSTRÖM and HULTMAN (1997) did not result in biopsy samples suitable for analysis due to artefacts caused by mechanical destruction of the muscle cells, we developed an alternative procedure by manually creating a vacuum on the modified *Bergström*-needle. One sample was used for histological examination to exclude that fat or connective tissue would affect measurements. The second sample was stored at -24 °C until further examination. The same procedure with the exception of local anesthesia was followed to obtain muscle biopsy samples for determination of potassium contents in muscle biopsies from third party animals (hospitalized cattle that had to be euthanized). Samples from third party animals were obtained immediately following euthanasia by intravenous administration of 60 mg/kg BW of a pentobarbital preparation (Euthadorm®, 400mg/ml, CP -Pharma, Burgdorf, Germany).

4.4.2 Sampling Scheme

4.4.2.1 Blood Sampling

Before and subsequently to the administration of the bolus, blood samples were obtained from the jugular vein. Whole blood samples were collected using Blutgas-Monovette® 2 ml LH (Lithium Heparin) collection tubes (no. 05.1147.020, Luer, 50 IU heparin/ml blood) for blood gas analysis, and S-Monovette® 9 ml LH (Lithium Heparin) collection tubes (no. 02.265, Luer, 16 IU heparin/ml blood) for determination of ionized potassium, sodium, chloride, calcium and levels of glucose and lactate. Plasma was harvested from heparinized whole blood within 30 minutes after sampling using Megafuge 3.0R centrifuge (Thermo Fisher Scientific GmbH, 63303 Dreieich, Germany) and used for analyses of bilirubin, albumin, total protein, blood urea nitrogen (BUN), creatinine, total calcium, magnesium, aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyltransferase (gamma-GT), inorganic phosphate, non-esterified fatty acids (NEFA) and blood urea nitrogen (BUN). Whole blood samples for determination of hematocrit (HCT), white blood cell counts (WBC), red blood cell counts (RBC) and platelet counts (PLT) were drawn into Monovette® 9 ml K3E (K3 EDTA) collection tubes (no. 02.267.001, Luer, 1,6 mg EDTA/ml blood). The time scheme below shows the time of sampling and the variables determined at each time point. Analysis of blood samples took place in the laboratory run by the clinic.

Hour 0 / preceding bolus administration

- Ionized sodium, potassium, chloride, calcium (ionized and total), blood gas analysis, glucose, lactate, creatinine, BUN, NEFA, β-hydroxybutyrate, albumin, bilirubin, AST, gamma-GT, AP, phosphate, total magnesium, HCT, WBC, RBC, PLT, differentials

Hours 1, 2, 3, 12-18 and approximately 36-40 hrs after initial bolus administration

- Ionized sodium, potassium, chloride, calcium (ionized and total), blood gas analysis, glucose, lactate, creatinine, HCT, WBC, RBC, PLT.

Day 5 (84 hrs after initial bolus administration)

- Ionized sodium, potassium, chloride, calcium (ionized and total), blood gas analysis, glucose, lactate, HCT, WBC, RBC, PLT, ß-hydroxybutyrate, NEFA, albumin, bilirubin, AST, gamma-GT, AP, phosphate, total magnesium, BUN, creatinine.

4.4.2.2 Urine sampling

At admission

- pH, glucose, bilirubin, ketones, protein, hemoglobin/erythrocytes semi quantitatively

4.4.2.3 Muscle biopsy sampling

Diagnostic biopsies of the vastus lateralis muscle were obtained for determination of the amount of connective tissue by histological examination in order to assure that pure biopsies of skeletal muscles were examined on potassium content. The biopsies were obtained preceding bolus administration (h0) and at final examination (approximately 84 hours following bolus administration or sham bolus application) in nine animals (3 cows of Group 1a; 4 cows of Group 2; 2 cows of control Group 1b).

4.4.3 Laboratory Analysis

Laboratory analyses of **blood samples**: blood gas analysis and determination of plasma levels of ionized potassium, sodium, chloride, calcium and levels of glucose and lactate was performed using heparinized whole blood with the Blood Gas Analyzer (ABL5, Radiometer Copenhagen, Bronshoj, Denmark) and ISE (EML 105 Analyzer, Radiometer Copenhagen, Bronshoj, Denmark). Whole blood from tubes with EDTA supplement were used for determination of hematocrit (HCT), white blood cell counts (WBC), red blood cell counts (RBC), platelet counts (PLT) by electronic cell counter (VetABC™, Scil, 68519 Viernheim, Germany). Differentials were determined by microscopic evaluation of blood smears that had been stained using May-Gruenwald-Giemsa staining. ß-hydroxybutyrate was determined by cowside test (Accucheck Aviva®; Roche Diagnostics, Basel, Switzerland). Analyses of bilirubin, albumin, total protein, blood urea nitrogen (BUN), creatinine, total calcium, magnesium, aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyltransferase (gamma-GT) and inorganic phosphate were performed from plasma using clinical chemistry analytical device (VetScan VS2TM; scil animal care company GmbH, Viernheim, Germany). Analysis of non-esterified fatty acids (NEFA), blood urea nitrogen (BUN) and creatinine was performed from plasma using automatic analyzer (Cobas Mira Automatic Analyzer, Roche Diagnostics GmbH, Basel, Switzerland).

Urine analysis was performed by visual examination and by use of a Combur 9 Test ® (Roche Diagnostics GmbH, Basel, Switzerland).

Muscle biopsy samples were sent to the laboratory of the Institute of Animal Nutrition, Freie Universität Berlin, Germany for determination dry matter and potassium content. For determination of dry matter content (DM) the samples were weighed (T1) and then dried for 4 hours at 103°C. After cooling down the samples were weighed again (T2) and dry matter content was computed.

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

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

$$RA\ (g/kg) = T2/T1 \times 1000$$

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) in the laboratory of the Institute of Animal Nutrition, Faculty of Veterinary Medicine, Freie Universität Berlin. 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.

Muscle biopsy samples for histologic examination were fixated in a 10 % formaldehyde solution for at least 24 hours before further processing. After dehydration with alcohol-water solutions and clearing of the samples of all residual alcohol the latter was replaced by melted paraffin. The paraffin-infiltrated tissue was then placed in fresh paraffin blocs and allowed to

cool. The embedded and cooled sample was cut with a microtome and placed on microscope slides. For histological examination of muscle tissue and possible findings of connecting tissue or residuals of fat cells as well as glycoproteins, glycolipids and mucins the samples were stained using the Periodic acid-Schiff method. Stained slides were examined using a light microscope and a digital slide scanner (ScanScope ®, Aperio ePathology, Leica Biosystems Nussloch GmbH, Nussloch, Germany) at the Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Freie Universität Berlin.

4.4.4 Clinical Examination

Clinical examination as described by ROSENBERGER (1990) in accordance with the daily routine of the clinic, as well as additional investigations described as follows, were performed on a daily basis starting at the day of admission until calendar day five of hospitalization and included evaluation of

- Appetence/feed intake
- Behavior
- Nutritional status
- Pulse and heart rate
- Arterial fill
- Pulse rhythm
- Respiratory rate
- Body temperature (rectal)
- Abdominal tension
- Rumen fill
- Rumen motility
- Presence of a steelband effect at percussion-auscultation in the left flank and/or positive ballottement indicative for LDA
- Liver percussion field (extension, pain)
- Rectal examination
- Composition of feces
- Evaluation of vaginal discharge by Metricheck™ (Hamilton 3242, Simcro, New Zealand) device
- Daily milk yield
- Evaluation of the position of the abomasum by ultrasound

Clinical examination was performed at hospitalization and was repeated in intervals of 20 to 24 hours (once daily in the morning until calendar day five following application of the bolus). Data of the clinical examination and treatments were recorded on separate data sheets for each patient. Clinical outcome and days of hospitalization were recorded.

Daily feed intake (kg WW) was evaluated by weighing the feedstuff (hay and concentrate separately) before supply and subsequently the remains in the trough. Subtraction of the remains from the provided amount of feed gave the daily feed intake in kg WW.

4.4.5 Evaluation of vaginal discharge with the Metricheck™ device

The vaginal discharge was evaluated using Metricheck™ device. The device is a stainless steel rod with a silicon hemisphere on one end. After cleaning of the vulva and disinfection of the device it was inserted up to the cranial extent of the vaginal fornix and subsequently

retracted caudally. Discharge remained in the latex cup and was then evaluated as referring to the description of PLETICHA et al. (2009) and LAMBERTZ et al. (2014): Score 0: translucent mucus. Score 1: mucus containing flecks of white or off-white pus. Score 2: less than 50% white or off-white mucopurulent material. Score 3: greater than 50% white or yellow pus that may be sanguineous. Score 4: lochia (< 21 DIM). Score 5: putrid material (e.g. due to retentio secundinarum).

4.4.6 Ultrasonographic assessment of change in abomasal position

The position of the abomasum was evaluated from the left and right flank using an ultrasound unit and a linear transducer at a wavelength of 3.5 MHz. The examination was performed using the technique described by BRAUN et al. (1997). The margins of the abomasum were identified as following: *prox*: the distance between the caudal aspect of the xiphoid process and the cranial abomasal margin. *dist*: the distance between the caudal aspect of the xiphoid process and the caudal abomasal margin. *ri-cran/le-cran*: the maximal right and left lateral extensions at the midpoint of the cranial half of the abomasum. *ri-caud/le-caud*: the maximal right and left lateral extensions at the midpoint of the caudal half of the abomasum (WITTEK, 2005) (Figure 7). In animals suffering from LDA only the maximal left lateral extension was measured in cm.

4.4.7 Surgery

Whenever LDA was diagnosed surgery was performed between 15 and 20 hrs following admission (next day) by right flank laparotomy, reposition of the abomasum and omentopexy following the Hannover approach (DIRKSEN, 1967).

4.5 Statistical Methods

All variables were evaluated and compared between treatment groups using appropriate descriptive statistics and graphics in collaboration with the department for Nonclinical Biostatistics of Boehringer Ingelheim Pharma GmbH & Co KG, BDM Europe. Repeated Measures ANOVA and pairwise comparisons with Tukey-Kramer Adjustment were performed for assorted variables. Significance tests were used where appropriate. The statistical analyses were conducted using the SAS System Version 9.2. Pearson product-moment correlation was performed for estimating the relationship between the concentration of potassium in plasma and muscle tissue and the effect of pH-values on plasma and muscle potassium concentrations using SPSS. Correlation coefficients were interpreted using the scale as provided by Salkind, with 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 being defined as very strong, strong, moderate, weak and very weak or no relationship, respectively (CHUNG, 2007). The level of significance was set at $p<0.05$.

5 RESULTS AND EVALUATION

5.1 Clinical Trial

5.1.1 Animals

In the period from August 2012 to July 2013, 47 cows submitted to the clinic by local practitioners were enrolled in the study. Four animals had to be withdrawn from the study as these met the exclusion criteria at a certain time point. Cows with moderate hypokalemia (Group 1a/1b: $[K^+] \geq 2.8$ and < 3.5 mmol/l) were averagely 15.2 (2-43) days postpartum, whereas cows with severe hypokalemia (Group 2: $[K^+] < 2.8$ mmol/l) had calved on average 18.1 (11-33) days before admission to the clinic. For three animals, no parturition dates were provided, one of which was 7 months pregnant at admission to the clinic (B130190, Group 1a).

5.1.2 Diagnoses in Cows with Hypokalemia

37 (78.7%) of the study animals were diagnosed with LDA at admission, 32 (68.1%) suffered from ketosis, two cows suffered from clinical mastitis (4.2% of all included animals) and 16 (34%) were diagnosed with metritis (Table 2). Noteworthy, LDA and ketosis was a comorbidity existing in 23 (48.9%) of study animals.

In four cows with LDA, abomasal displacement was no longer present on day two of hospitalization. One of these animals belonged to Group 2 (B130042), one to Group 1a (B1300040) and two to the untreated controls (Group 1b; B130154, B130079).

Table 2. Diagnosis in hypokalemic cows at admission.

	Group							
	1a (19)		1b (17)		2 (11)		All (47)	
	N	%	N	%	N	%	N	%
Diagnosis at admission								
Ketosis	**13**	68.4	**13**	76.5	**6**	54.5	**32**	68.1
LDA	**16**	84.2	**14**	82.4	**7**	63.6	**37**	78.7
Mastitis	**0**	0.0	**1**	5.9	**1**	9.1	**2**	4.2
Metritis	**5**	26.3	**6**	35.3	**5**	45.5	**16**	34

The clinical outcome is shown in Table 3. Six cows from 47 (12.8%) had to be euthanized, all but one after completion of the study. Four cows were excluded due to rescue treatment.

Table 3. Outcome of study animals.

	Group							
	1a (19)		1b (17)		2 (11)		All (47)	
	N	%	N	%	N	%	N	%
Outcome								
Left hospital	**18**	94.7	**15**	88.2	**8**	72.7	**41**	87.2
Euthanized	**1**	5.3	**2**	11.8	**3***	27.3	**6**	12.8
Excluded	**0**	0	**1**	5.9	**3**	27.3	**4**	8.5

*One cow was euthanized on day three of the study

5.1.3 Feed Intake

Feed intake in kg WW was measured for all study animals beginning with the first feeding after arriving at the clinic. Discrepancies in the number of animals for which feed intake could be recorded arise from the differences in the time of arrival on day one.

An increased intake of concentrate, hay, and concentrate plus hay was observed in animals from all groups in the days following hospitalization (Figure 2, Figure 3). Changes in wet matter intake did not differ significantly between the Groups 1a, 1b and 2. Furthermore, there was no difference in intake of kg WW between the animals of Group 1a either receiving one, or two boluses.

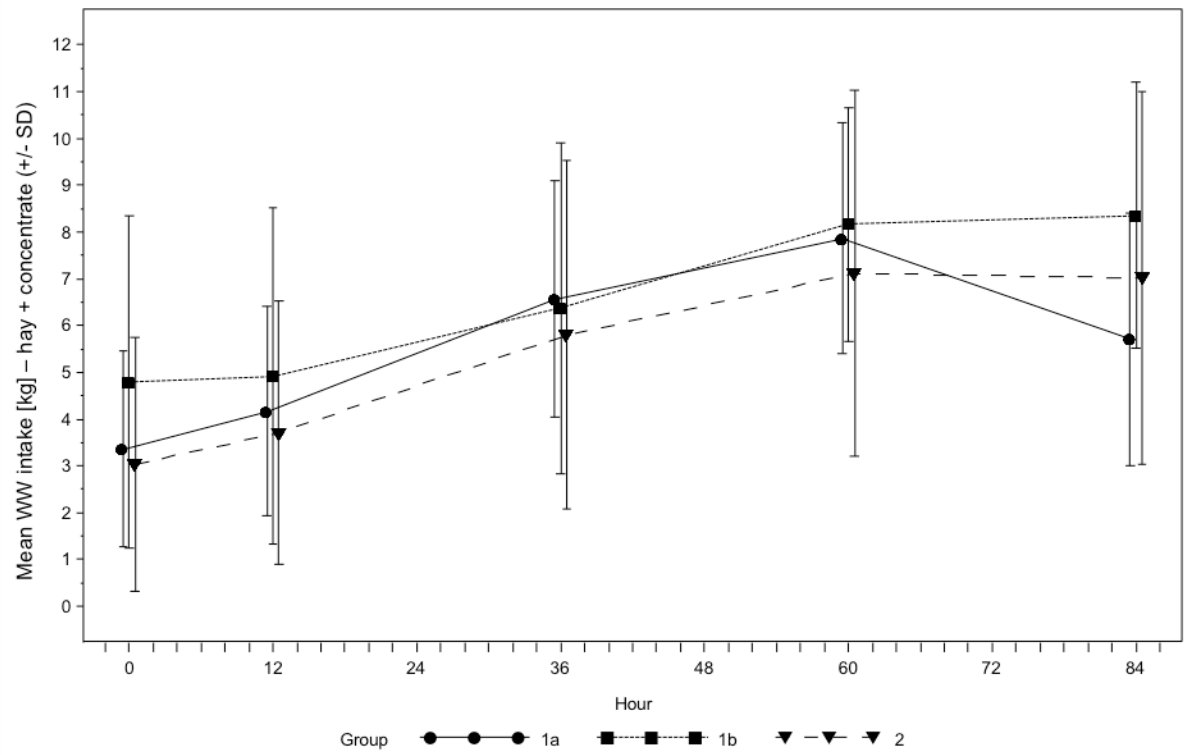

Fig. 2. Mean feed intake [kg ± SD] in cows with hypokalemia. The figure represents the mean feed intake (±SD) of hay and concentrate [kg WW] following potassium bolus administration (Group 1a and Group 2) and sham administration (Group 1b).

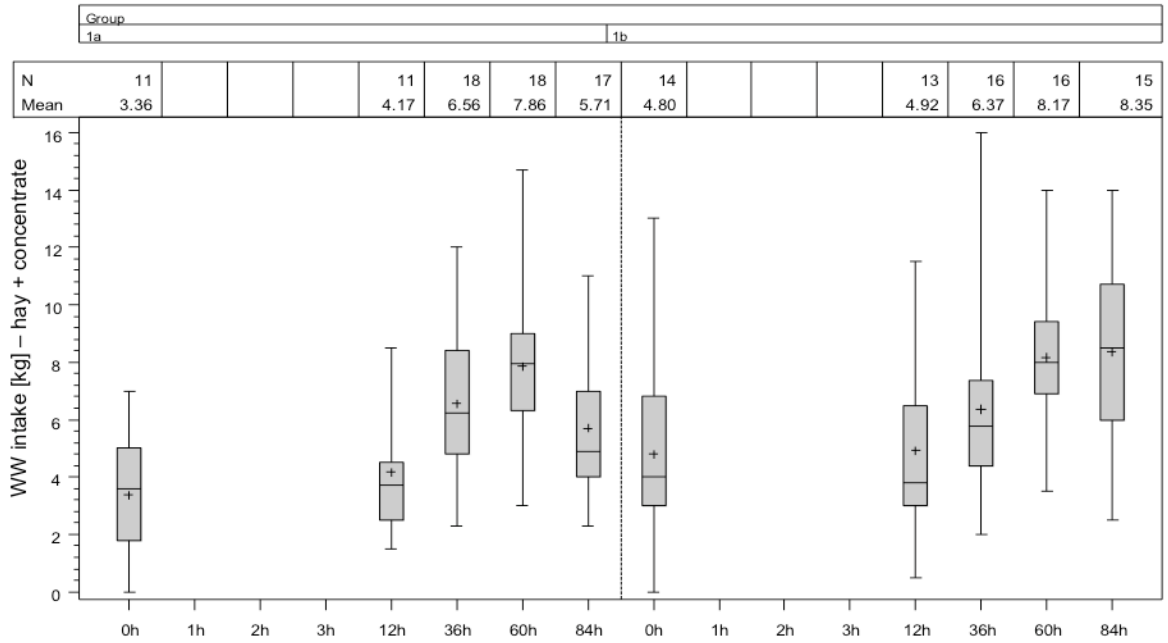

Fig. 3. Boxplots representing feed intake (kg WW) in two groups of cows with hypokalemia. Results are presented for feed intake before (0 hrs) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.4 Rumen Motility

Ruminal movements defined as the number of ruminal contractions audible within three minutes of auscultation increased in animals from all groups with time from the day of hospitalization. When evaluated over the whole observation period the increase did not differ between groups. Descriptive analysis show that animals belonging to Group 2 had least ruminal contractions at 0 hrs (mean 1.2 contractions in 3 minutes) and a mean increase of 1.0 contractions per three minutes over the time span of 84 hours. Group 1a showed a mean increase in the frequency of ruminal contractions of 1.5 contractions per three minutes and Group 1b showed a mean increase of 0.1 contractions within three minutes over the time span of 84 hours, however, noteworthy, mean ruminal contractions were highest in the control group (1b) at 0 hrs (Figure 4, Table 4).

Figure 4 represents the number of ruminal movements (±SD) that were audible in the left flank at auscultation over a time span of three minutes in cows following potassium bolus administration (Group 1a and Group 2) and sham administration (Group 1b).

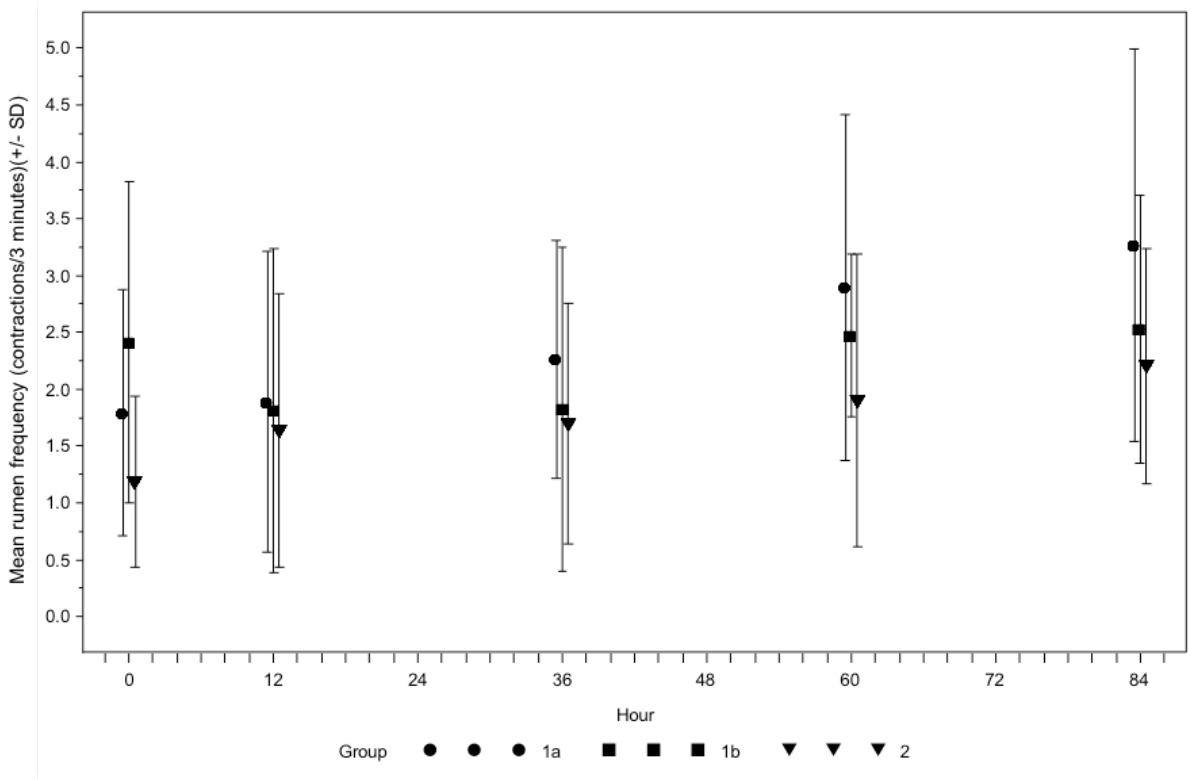

Fig. 4. Ruminal movements as number of contractions over 3 minutes audible in cows with hypokalemia at different time points after feeding of cows receiving potassium bolus administration (Group 1a and Group 2) and sham administration (Group 1b).

Table 4. Descriptive statistics of ruminal movements as number of contractions that were audible in the left flank at auscultation over a time span of three minutes in cows with hypokalemia following potassium bolus administration (Group 1a/Group2) and sham administration (Group 1b).

		Min	Max	Mean	SD
Group	Hour				
1a	0	0.0	4.0	1.79	1.084
	12	0.0	4.0	1.89	1.323
	36	0.0	4.0	2.26	1.046
	60	0.0	8.0	2.89	1.524
	84	1.0	8.0	3.26	1.727
1b	0	0.0	3.0	2.41	1.417
	12	0.0	2.0	1.81	1.424
	36	0.0	2.0	1.82	1.425
	60	1.0	2.0	2.47	0.171
	84	0.0	3.0	2.53	1.179
2	0	0.0	2.0	1.18	0.759
	12	0.0	3.0	1.64	1.206
	36	0.0	3.0	1.70	1.059
	60	0.0	3.0	1.90	1.287
	84	0.0	3.0	2.20	1.033

5.1.5 Milk Yield

There were no significant differences between treatment groups with respect to daily milk yield during the observation period (Figure 5, Figure 6). However, AUC was used as an additional outcome measure and divided by time to account for the fact that the milk yield was measured only at milking times following the clinic's protocol (mornings and afternoons) as opposed to fixed time points before or after bolus administration. It is worthy of note, that observation period for milk yield began on day 2 of hospitalization, as cows arriving later in the evening at the clinic were not milked then. Time-adjusted AUC of milk yield in liters per day showed that milk yield (l/d) of Group 2 stayed markedly below milk yield (L/d) of Groups 1a and 1b, and that Group 1b had a slightly lower milk yield (l/d) than Group 1a. Figure 5 represents the milk yield in liters per day (± SD) of cows following potassium bolus administration (Group 1a and Group 2) and sham administration (Group 1b).

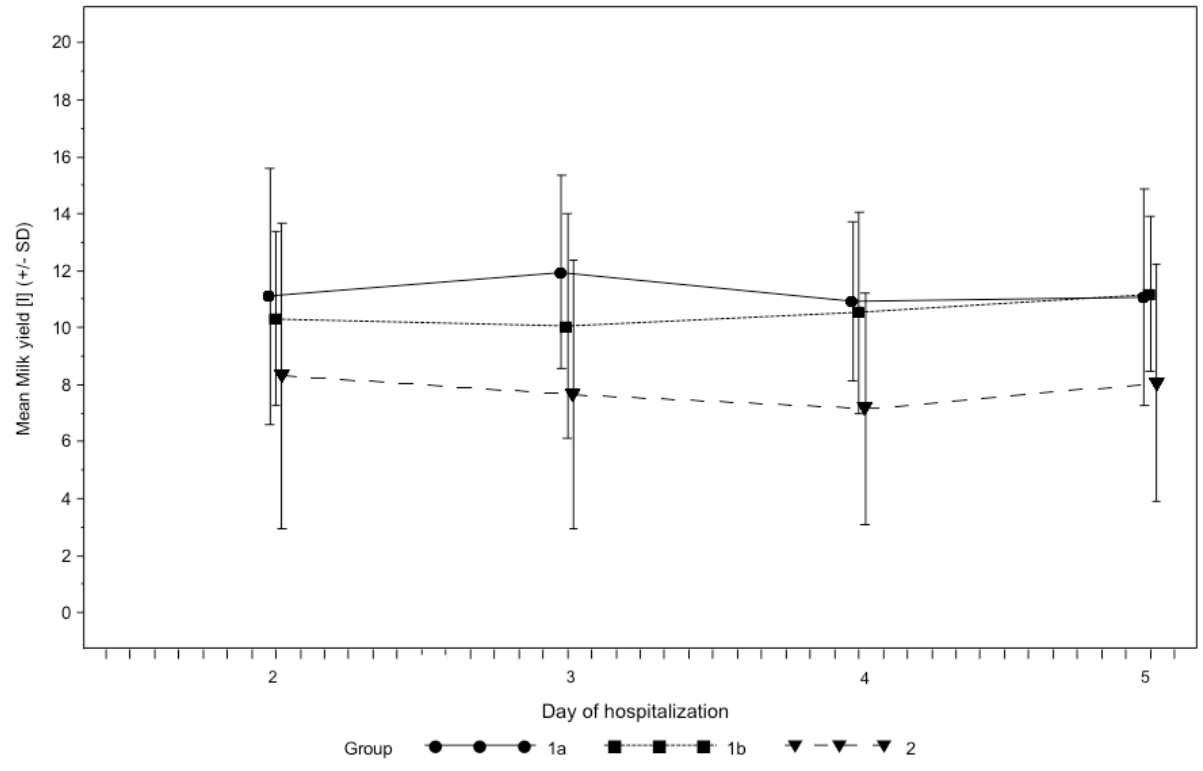

Fig. 5. Mean time course of milk yield in liters [l ± SD] of cows with hypokalemia. The graph illustrates the mean daily milk yield (mornings and evenings) of study animals in liters (± SD) computed over the time span of the observation period starting on day 2. Results are presented for animals following potassium bolus administration (Group 1a) and sham administration (Group 1b).

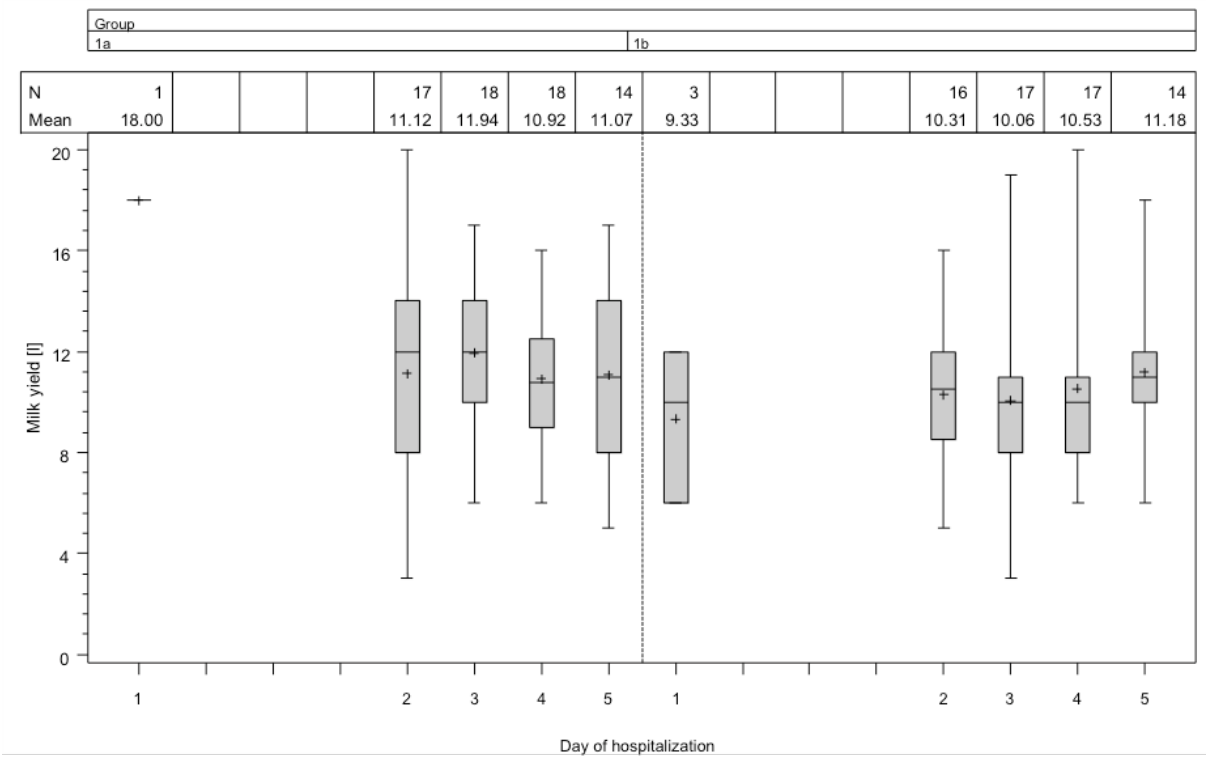

Fig. 6. Boxplots representing milk yield in liters in two groups of cows with hypokalemia computed over the time span of the observation period. Results are presented for animals following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.6 Position of Abomasum

Ultrasonographic examination of the abdomen was performed in all animals upon arrival in the clinic. Of the 47 cows enrolled in the study, only ten subjects had not been diagnosed with LDA at admission to the clinic and in one other animal the dislocation of the abomasum was no longer present at the initial ultrasonographic examination on day one of the clinical trial (B130169, Group 1b). However, of these eleven cows, only eight were suitable for proper and complete ultrasonographic examination of the abomasum in its physiological position (Table 5), as one subject had to be euthanized before completion of the study (B120293) and in two other cows massive abdominal fat (B120288) and presumed pneumoperitoneum due to liver biopsy (B120272), respectively, hindered the acquisition of reliable data. In the eight animals undergoing complete ultrasonographic evaluation, no marked changes in the position and dimensions of the abomasum occurred over the period of the study. The mean measured length of the abomasum, calculated as the difference between cranial and caudal margins, ranged from 19.1 cm to 25.4 cm. The mean lateral extension to the right ranged from 9.1 cm to 14.1 cm in the cranial region (approximately at first quartile of length) and 22 cm and 25.4 cm in the caudal region (approximately at third quartile of length). The mean lateral extension to the left was measured as 6.1 cm – 10.3 cm (cranial) and 6.6 cm – 12.3 cm (caudal) (Table 5, Figure 8).

According to standard operating procedures of the clinic, patients suffering from LDA underwent corrective surgery within the first 24 hours following admission, usually the morning after initial hospitalization (Day 2). In order to be able to examine the abdominal cavity including internal organs and to ensure the best possible outcome for study animals, surgery was performed by right flank laparotomy following the Hannover approach (DIRKSEN, 1967). As laparotomy inevitably involves influx of air into the abdominal cavity, the resulting iatrogenic pneumoperitoneum eliminated the possibility of ultrasonographic assessment of the abomasum after surgery and the analysis of changes of the abomasal position over time in said animals.

Figure 8 schematically shows the change in abomasal position as determined by ultrasonography in treated animals (B120317 (Group 2), B130209 (Group 2), B130224 (Group 2) and B130229 (Group 1a) as well as controls (B130003, B130169, B130185 and B130228) (all Group 1b). The gray squares represent the position of the margins of the abomasum as shown in Figure 7 with the lightest shade on the gray scale representing measurement at admission and the darkest shade representing measurement on calendar day 5 (after 84 hours). Table 5 shows mean values (cm ± SD) of measurements at different time points. There were no statistically significant differences between treatment groups and the control group in terms of position of the abomasum with time as determined by ultrasound. As described by other authors (BRAUN et al., 1997; WITTEK et al., 2005), the abomasum was located along the ventral abdominal wall with a greater lateral extension to the right of the linea alba.

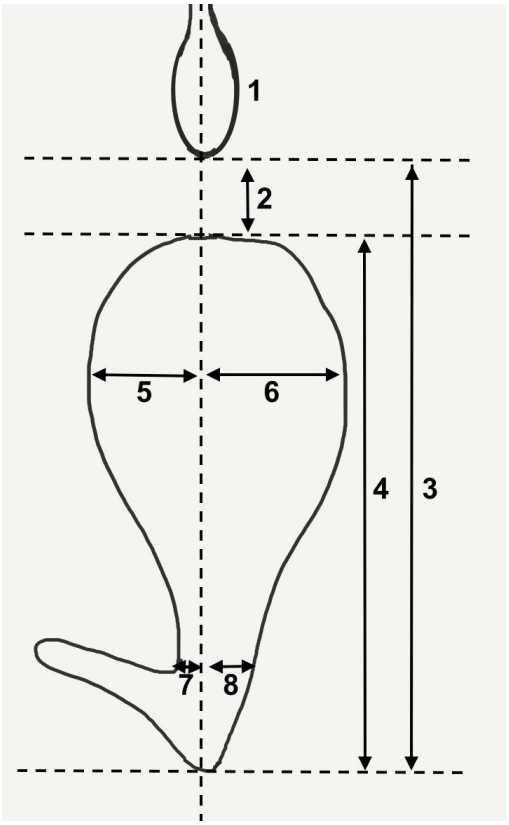

Fig. 7. Ultrasonographic assessment of the abomasum of the cow, sagittal section from a dorsal view. 1 Xiphoid process, 2 Extension from xiphoid process to cranial margin of abomasum *(prox)*, 3 Extension from xiphoid process to caudal margin of abomasum *(dist)*, 4 Length of abomasum, 5 Left lateral extension of abomasum in cranial region measured from the linea alba *(le-cran)*, 6 Right lateral extension of abomasum in cranial region measured from the linea alba *(ri-cran)*, 7 Left lateral extension of abomasum in caudal region measured from the linea alba *(le-caud)*, 8 Right lateral extension of abomasum in caudal region measured from the linea alba *(ri-caud)*. Dotted vertical line represents linea alba (modified from BRAUN et al. (1997)).

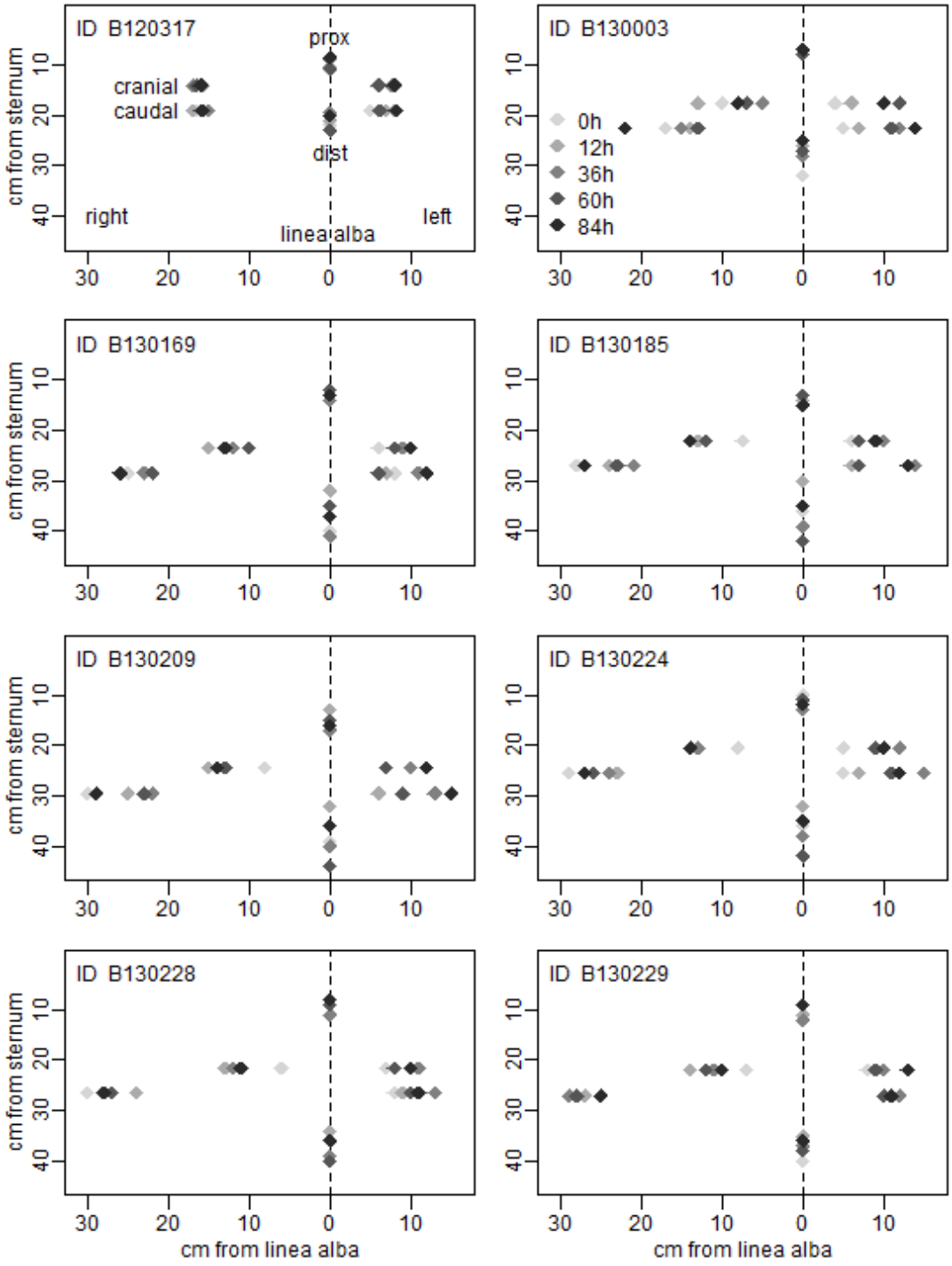

Fig. 8. Change of abomasal position in cows that did not undergo surgery from hour 0 to hour 84 following oral treatment with the bolus formulation as determined by ultrasonography (*prox*: the distance between the caudal aspect of the xiphoid process and the cranial abomasal margin. *dist*: the distance between the caudal aspect of the xiphoid process and the caudal abomasal margin. *Right cranial/left cranial*: the maximal right and left lateral extensions at the midpoint of the cranial half of the abomasum. *Right caudal/left caudal*: the maximal right and left lateral extensions at the midpoint of the caudal half of the abomasum) (see also 4.4.6). Dotted vertical line represents linea alba. Squares represent position of margins of t. abomasum as shown in Figure 7 with lightest shade on gray scale representing measurement at admission and darkest shade representing measurement on calendar day 5.

Table 5: Results of ultrasonographic examination of the abomasum in 8 cows with hypokalemia at different time points. Values are given as mean (cm) ± SD.

	Time point of measurement				
Measurement	0h	12h	36h	60h	84h
Prox	10.8 ± 2.5	11.2 ± 2.1	12.3 ± 3.2	11 ± 2.3	11.1 ± 3.4
Dist	35.6 ± 6.2	30.3 ± 4.6	35.2 ± 7.5	36.4 ± 7.6	32.5 ± 6.3
Length	24.9 ± 6.5	19.1 ± 4.6	22.9 ± 5.5	25.4 ± 6.8	21.4 ± 5.3
Le-cran	6.1 ± 1.2	8.9 ± 1.5	10 ± 1.3	8.3 ± 1.8	10.3 ± 1.6
Le-caud	6.6 ± 1.8	7.5 ± 1.7	12.3 ± 2.1	8.8 ± 2.1	12 ± 2.1
Ri-cran	9.1 ± 3.1	14.1 ± 1.8	12 ± 3.4	12 ± 2.8	12.5 ± 2.6
Ri-caud	25.4 ± 5.6	22 ± 4.3	22 ± 5	22.3 ± 5.3	25 ± 4.3

(*prox*: the distance between the caudal aspect of the xiphoid process and the cranial abomasal margin. *dist*: the distance between the caudal aspect of the xiphoid process and the caudal abomasal margin. *Length:* difference between *Prox* and *Dist. Right cranial/left cranial*: the maximal right and left lateral extensions at the midpoint of the cranial half of the abomasum. *Right caudal/left caudal*: the maximal right and left lateral extensions at the midpoint of the caudal half of the abomasum)

In four cows with LDA, abomasal displacement was no longer present on the day following hospitalization (spontaneous reposition). One of these animals belonged to Group 2 (B130042), one to Group 1a (B1300040) and two to the untreated controls (Group 1b; B130154, B130079). Noteworthy, in B130079, abomasal displacement recurred on hospital day 3. In order to prevent recurrence, all animals with spontaneous reposition of DA still underwent surgery in order to prevent reoccurrence of DA, which hindered further ultrasonographic examination of the abdominal cavity due to free air accumulation (pneumoperitoneum) as explained above. No differences were found with respect to spontaneous resolution of LDA within the first 12 to 24 hours of hospitalization in the different groups, nor did the extent of the abomasum as determined by ultrasound before and following the administration of the potassium bolus and the sham administration decrease significantly.

5.1.7 Evaluation of vaginal discharge by Metricheck™

PLETICHA et al. (2009) described the diagnostic technique for clinical endometritis for cows at 21 DIM or more using the Metricheck™ device. In the present study, evaluation of vaginal discharge of dairy cows from 21 DIM onwards and at 84 hrs following hospitalization was performed in order to find out if potassium supplementation would enhance the self-cleaning mechanism of the uterus characterized by improvement/reduction in score number. Cows in the puerperal phase (less than 21 DIM) were examined as well; however, physiological discharge (lochia) hindered comparison between individual animals, which is why category changes were only analyzed for animals of > 21 DIM (n = 12). The change of score categories over 84 hours was analyzed using the Kruskall-Wallis-Test and there were no significant differences observed between treatment groups (Group 1a/Group 2) and untreated controls (Group 1b) (Table 6).

Table 6. Changes in vaginal discharge characteristics (improvement/reduction in score number) as evaluated with Metricheck™ device from 0h to 84h of hospitalization for 12 hypokalemic dairy cows > 21 DIM.

	Number of Metricheck™ Categories Change			
	0	1	2	All
Number of animals in				
Group 1a	1	3	0	4
Group 1b	1	3	1	5
Group 2	2	1	0	3

0 = no change; 1 = improvement/reduction in score number over one category; 2 improvement/reduction in score number over two categories

5.1.8 Laboratory Analysis

5.1.8.1 Plasma Potassium Levels

Animals from all groups showed an increase in plasma potassium concentrations with time during the observational period (Figure 9). Mean and median plasma potassium levels in Group 1a returned to normal which means a return to levels within the limits of the reference range within one hour after single bolus application, whereas this was observed for animals of the (untreated) control group (Group 1b) at 36 hours. For Group 2 the median plasma potassium concentration was within the reference range at 12 hours (before administration of the second bolus) and mean potassium concentration reached the reference range at 36 hours following application of the first (of two or three) boluses.

The overall change in plasma potassium concentrations was significantly different between treatments ($p < 0.01$) and differed between treatments over time ($p < 0.01$). The time effect was significant ($p < 0.01$), i.e. the plasma potassium level of the groups changed with time. Pairwise comparisons with Tukey-Kramer-Adjustment showed highly significant differences in the alterations in plasma potassium concentrations between Groups 1a and 1b ($p < 0.01$) and Group 1b versus Group 2 ($p < 0.01$).

The onset of the increase in plasma potassium levels occurred much earlier in treated animals when compared to untreated controls and was steeper than in animals from the control group. In non-treatment Group 1b plasma potassium concentrations increased with time; both median and mean potassium levels, however, exceeded threshold levels (lower border of the reference range) not earlier than at 36 hours after sham bolus administration (3.69 mmol/l and 3.6 mmol/l, respectively) (Figure 9). The mean increase in potassium levels was 0.6 mmol/l for Group 1a, 0.4 mmol/l for Group 1b and 1.22 mmol/l for Group 2.

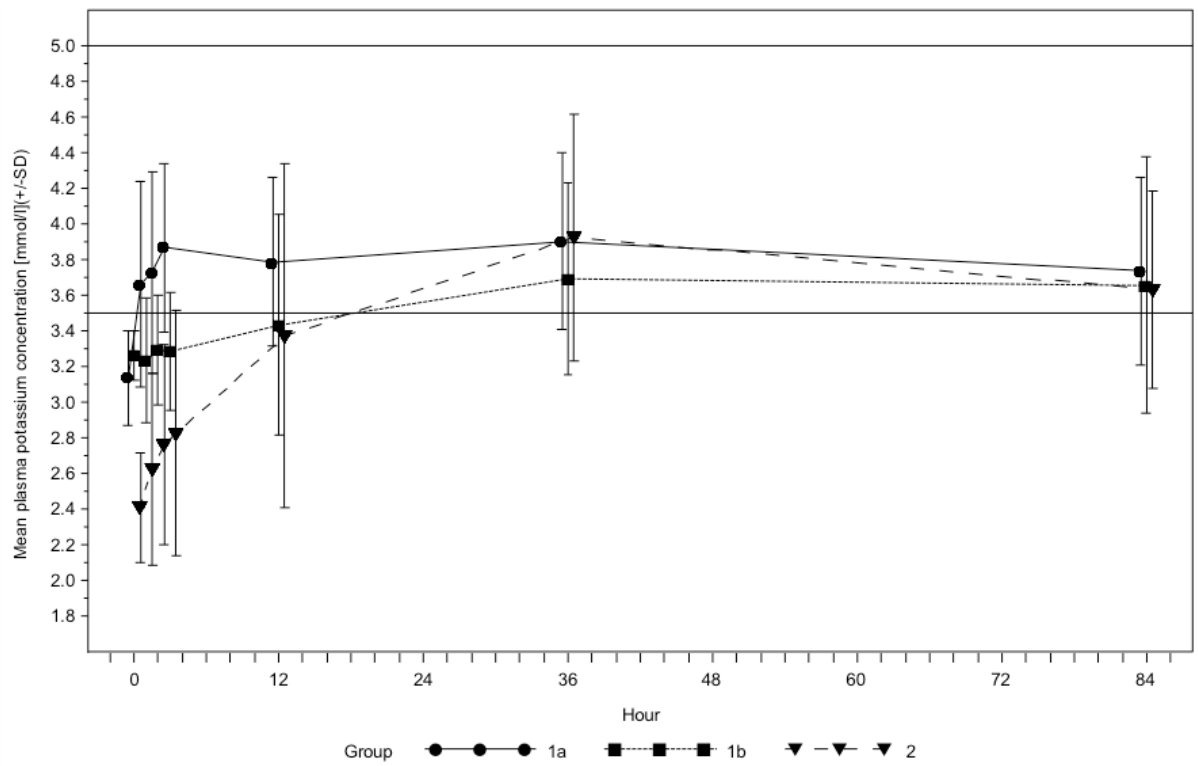

Fig. 9. Time course of mean plasma potassium concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at different time points following bolus administration (Group 1a, Group 2) and sham administration (Group 1b). The line indicates the lower border of the reference range.

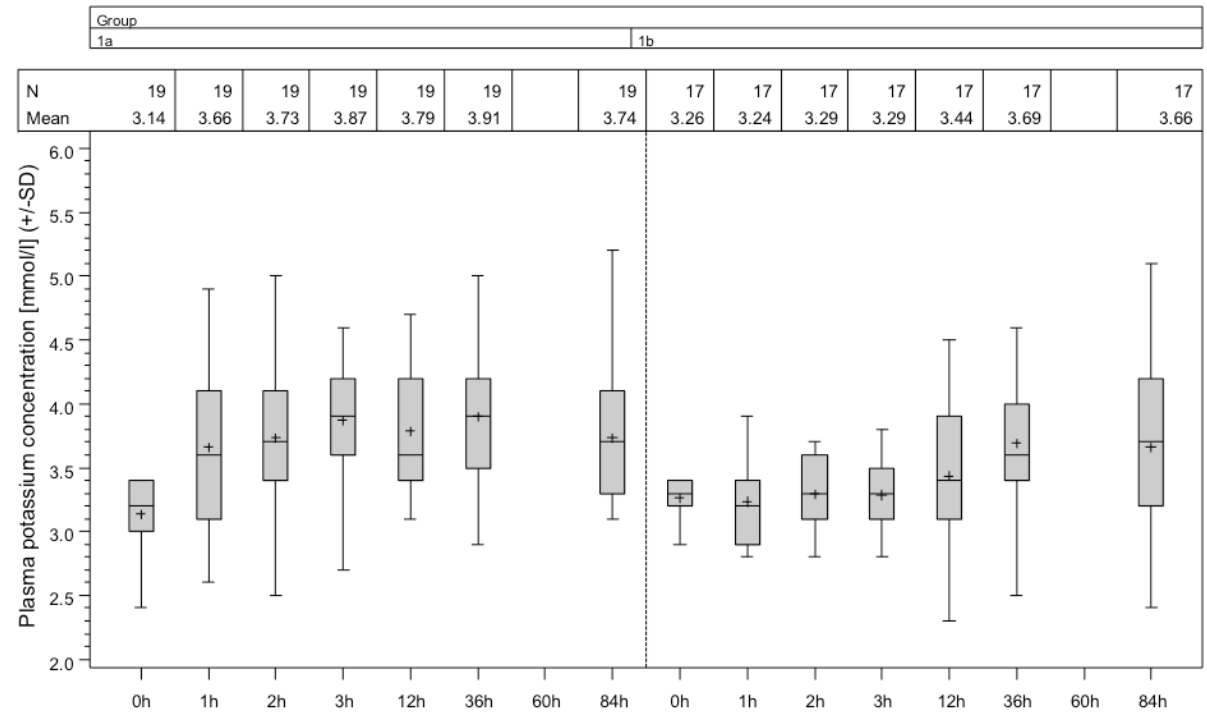

Fig. 10. Boxplots representing plasma potassium concentrations [mmol/l] in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.2 Potassium Content in Muscle Biopsies

5.1.8.2.1 Potassium content in muscle biopsies from third party animals

A total of 13 plasma and muscle biopsy samples obtained from 13 animals of German Holstein breed admitted to the clinic were included into our statistical evaluation, which originated from the work of EL-ZAHAR (2015), in order to study the relationship between plasma and muscle potassium contents in cattle. The ages ranged from 1 month to 2 years. The cattle suffered from various non-metabolic disorders and had to be euthanized due to unfair prognosis. Potassium concentrations in plasma were 4.01 ± 0.72 mmol/l and in muscle biopsy samples 89.59 ± 11.87 mmol/kg WW, respectively. The values of pH, pCO_2, HCO_3^- and BE 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 7).

Table 7. Potassium concentrations in both plasma and muscle biopsy samples and results of blood gas analysis in 13 third party animals. Results are expressed as mean values ± SD as reported by EL-ZAHAR (2015).

	N	Minimum	Maximum	Mean ± SD
pH-values	13	7.2	7.48	7.38 ± 0.07
pCO_2 (mmHg)	13	39	61	48.92 ± 6.38
HCO_3^- (mmol/l)	13	18	31	27.92 ± 3.33
BE (mmol/l)	13	-10	6	2.92 ± 4.11
Plasma potassium concentration (mmol/l)	13	2.5	4.9	4.01 ± 0.72
Muscle potassium concentration (mmol/kg)	13	72	108.93	89.59 ± 11.87

Plotting the results of potassium concentrations in muscle tissue biopsy samples against the results of plasma potassium concentrations revealed a very weak correlation between the muscle potassium contents and the plasma potassium concentrations (r = 0.06) (Figure 11).

A weak correlation was observed between plasma pH-values and plasma potassium levels (r = 0.32), while no correlation was observed between plasma pH-values and muscle potassium contents (r = 0.01) (Table 8).

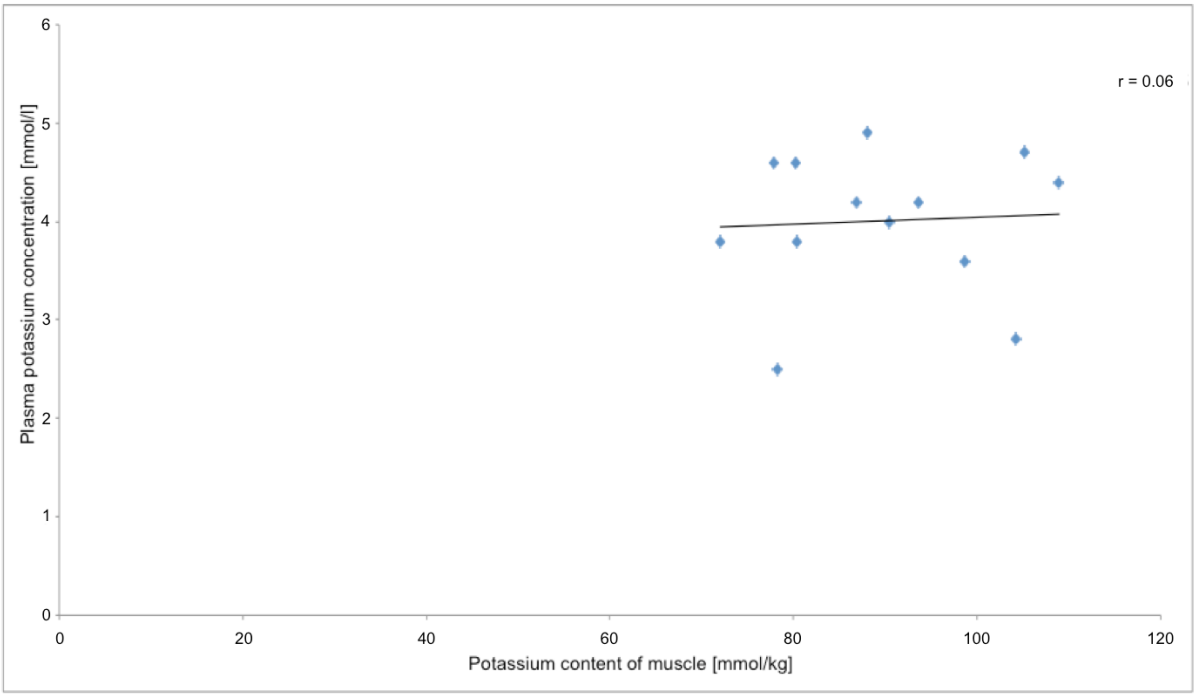

Fig. 11. Correlation between potassium concentrations in muscle biopsy samples and plasma samples of 13 cattle. r = Pearson correlation coefficient.

Table 8. Relationship between parameters of acid base metabolism and the concentration of potassium in plasma and muscle biopsy samples from 13 cows. Results are expressed by Pearson correlation coefficient and the significance, r (p value). r = Pearson correlation coefficient. The asterisk * = significance at $p < 0.05$ (EL-ZAHAR, 2015).

	Plasma potassium concentration (mmol/l)	Muscle potassium concentration (mmol/kg)
pH-value	0.32 (0.287)	0.01 (0.967)
pCO2 (mmHg)	0.39 (0.191)	0.34 (0.258)
HCO3- (mmol/l)	0.63 * (0.021)	0.32 (0.279)
BE (mmol/l)	0.6 * (0.032)	0.29 (0.338)
Muscle potassium concentration (mmol/kg)	0.06 (0.850)	-

5.1.8.2.2 Potassium content in muscle biopsies from study animals

Potassium levels in biopsies obtained from nine animals (three belonging to treatment Group 1a; four belonging to treatment Group 2 and two belonging to control Group 1b) from the vastus lateralis muscle demonstrated great differences between the two different times of sampling (Table 9).

Examination of microscopic slides of muscle biopsy samples with PAS reaction staining showed, that the muscle fibers obtained via suction assisted muscle biopsy were undamaged and homogenous. Presence of connecting tissue was minimal and concentrated on the

fringe area of the samples. The examination proved no disconnection in between muscle fibers and no other cell types except a low number of erythrocytes in the fringe areas could be identified.

Table 9. Plasma potassium concentrations [mmol/l] and muscle potassium concentrations [mmol/kg] in cows treated with a potassium bolus formulation and controls. Data from cows treated with potassium bolus formulation are presented in bold letters.

No	Date 1	K+ plasma (1) [mmol/l]	K+ muscle (1) [mmol/kg]	Date 2	K+ plasma (2) [mmol/l]	K+ muscle (2) [mmol/kg]
1(1a)	**14.05.13**	**3**	**86.09**	**18.05.13**	**3.1**	**77.08**
2(1b)	27.05.13	3.3	92.05	29.05.13	4.2	98.47
3(1a)	**27.05.13**	**3**	**105.35**	**31.05.13**	**4.3**	**80.33**
4(2)	**31.05.13**	**2.7**	**71.61**	**04.06.13**	**4**	**91.94**
5(2)	**14.06.13**	**2.6**	**85.06**	**18.06.13**	**3.2**	**62.33**
6(2)	**24.06.13**	**1.9**	**106.75**	**28.06.13**	**2.8**	**71.84**
7(2)	**26.06.13**	**2.1**	**67.14**	**30.06.13**	**3.5**	**36.8**
8(1b)	28.06.13	3.4	88.21	02.07.13	5.1	96.32
9(1a)	**28.06.13**	**3.3**	**78.18**	**02.07.13**	**5.2**	**99.26**

Pearson's correlation was used to estimate the relationship between plasma and muscle potassium content before and after receiving the bolus. No correlation between plasma and muscle potassium content was calculated for samples obtained before potassium supplementation, while a moderate correlation was found between plasma and muscle potassium contents in samples obtained after oral supplementation which was not statistically significant (Table 10). These results support the findings in third party animals presented above. In addition, the results of paired sample t-test showed that there was an improvement in extracellular plasma potassium concentrations after therapy with a significant increase in plasma potassium by 1.07 mmol/l (Table 11). Muscle potassium contents, however, did not show an increase following oral supplementation when compared to initial values.

These results show that the changes in plasma potassium concentrations occur independently from changes in muscle potassium concentrations. Significant increases in the plasma potassium concentration as observed following bolus treatment did not coincide with increases in muscle potassium contents. This is underlined by the fact that oral supplementation of potassium did not result in significant changes in muscle potassium contents.

Table 10. Correlation between potassium contents in muscle biopsy samples and plasma before and after oral supplementation with the potassium bolus formulation in seven hypokalemic cows. Results are expressed by Pearson correlation coefficient and the significance, r (p value). r = Pearson correlation coefficient.

	Plasma potassium before suppl.	Plasma potassium after suppl.
Muscle potassium before suppl.	-0.088 (0.851)	
Muscle potassium after suppl.		0.589 (0.164)

Table 11. Plasma and muscle potassium contents before and after oral supplementation with the potassium bolus formulation in seven hypokalemic cows, as well as paired sample t test comparing mean plasma and muscle potassium contents before and after oral potassium administration.

			Paired sample t test	
	Mean ± SD	Min. - Max.	Mean diff.	p value.
Plasma potassium before suppl. (mmol/l)	2.66 ± 0.51	1.9 - 3.3	1.07	0.003
Plasma potassium after suppl. (mmol/l)	3.73 ± 0.83	2.8 - 5.2		
Muscle potassium before suppl. (mmol/kg)	85.7 ± 15.4	67.1 - 106.8	-11.51	0.241
Muscle potassium after suppl. (mmol/kg)	74.2 ± 20.6	36.8 - 99.3		

5.1.8.3 Energy Metabolism – Non-esterified fatty acids (NEFA)

Repeated measures ANOVA showed that plasma NEFA concentrations decreased markedly, but not significantly, in all groups with time (Figure 12, Figure 13). Pairwise comparisons with Tukey-Kramer-Adjustment showed no significant differences in the changes of NEFA concentrations between groups. Mean decrease was 0.81 mmol/l in Group 1a, 0.87 mmol/l in Group 2 and 0.54 in control Group 1b.

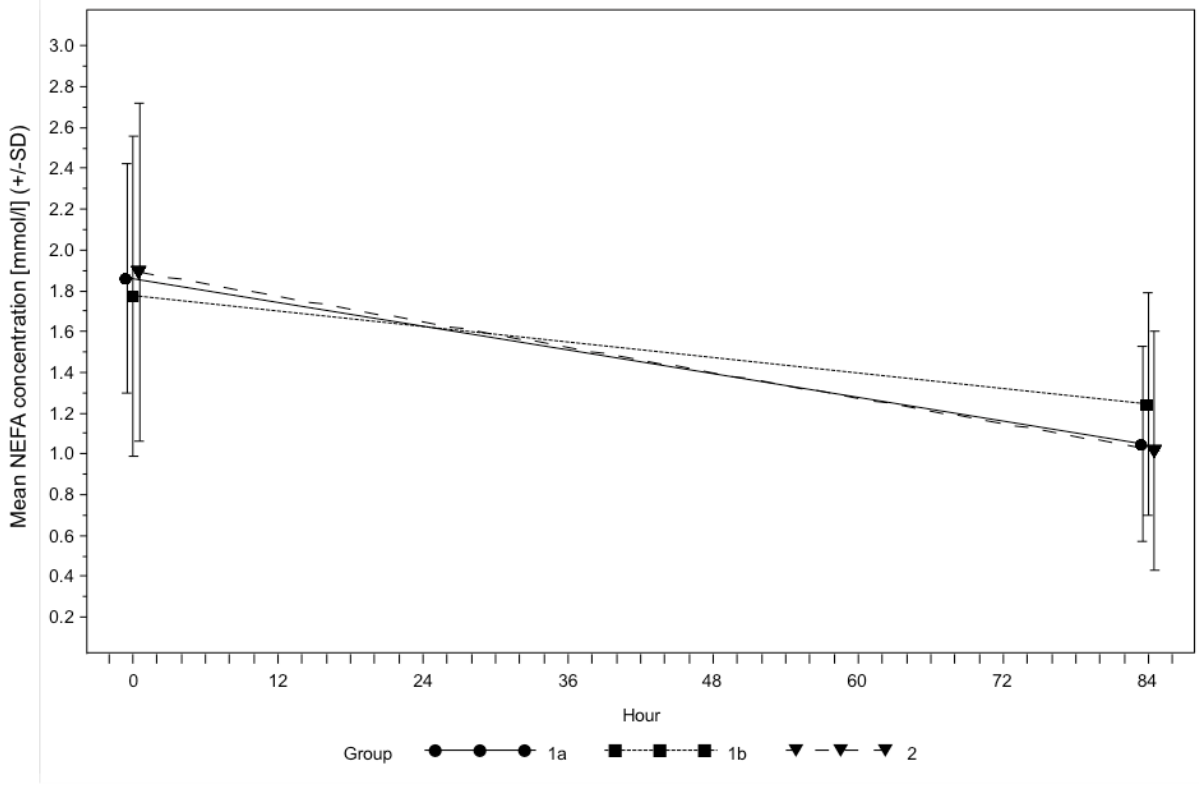

Fig. 12. Time course of mean NEFA concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at 84 hours following bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

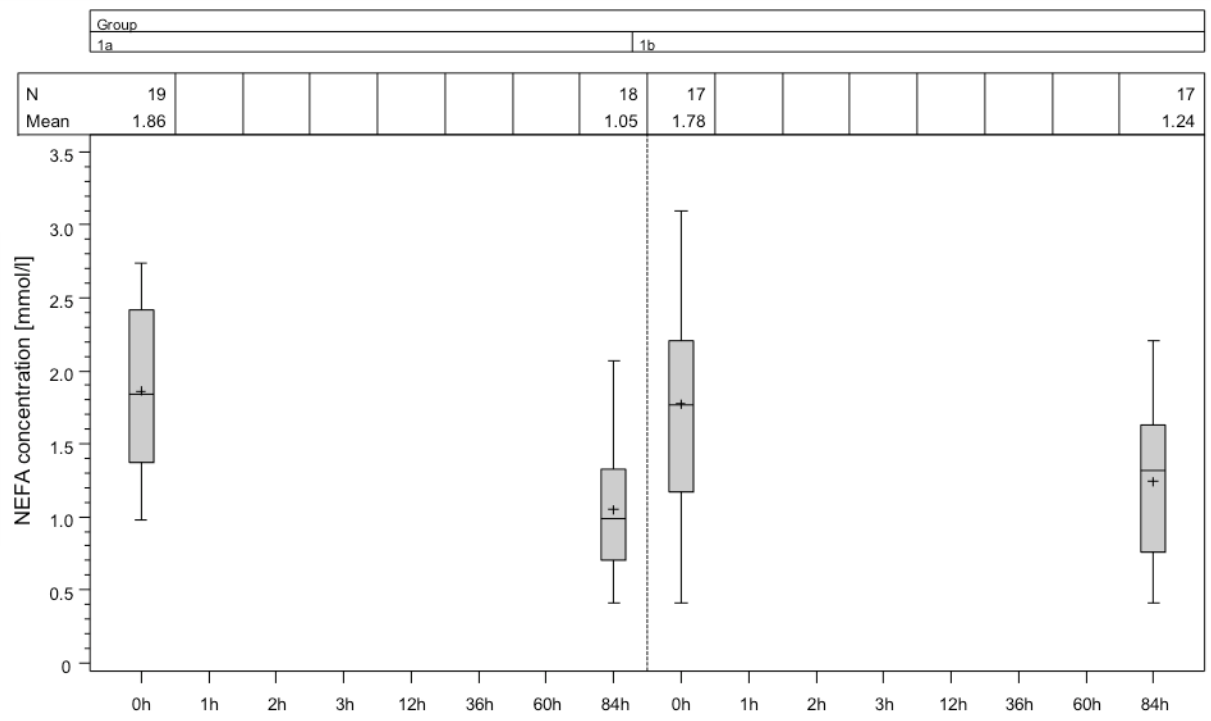

Fig. 13. Boxplots representing NEFA concentrations [mmol/l] in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and 84 hours following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.4 Energy Metabolism – Beta-hydroxybutyric acid (BHBA)

As for NEFA levels, the plasma concentrations of BHBA decreased markedly, but were not significantly different for all groups (Figure 14, Figure 15). Mean total decrease of BHBA was 0.81 mmol/l in Group 1a, 0.86 mmol/l in Group 2 and 0.36 in non-treatment controls (Group 1b). At admission 13 cows suffered from subclinical ketosis (BHBA levels of these animals ranged between 1.2 and 3 mmol/l), whereas in 19 cows the BHBA levels exceeded 3.0 mmol/l, a level that is considered as clinical ketosis. 84 hours following bolus administration or sham administration, respectively, BHBA levels in 14 cows still were above the level of 1.2 mmol/l indicative for subclinical ketosis and in nine cows the limit of 3.0 mmol/l BHBA as indicative for clinical ketosis was passed.

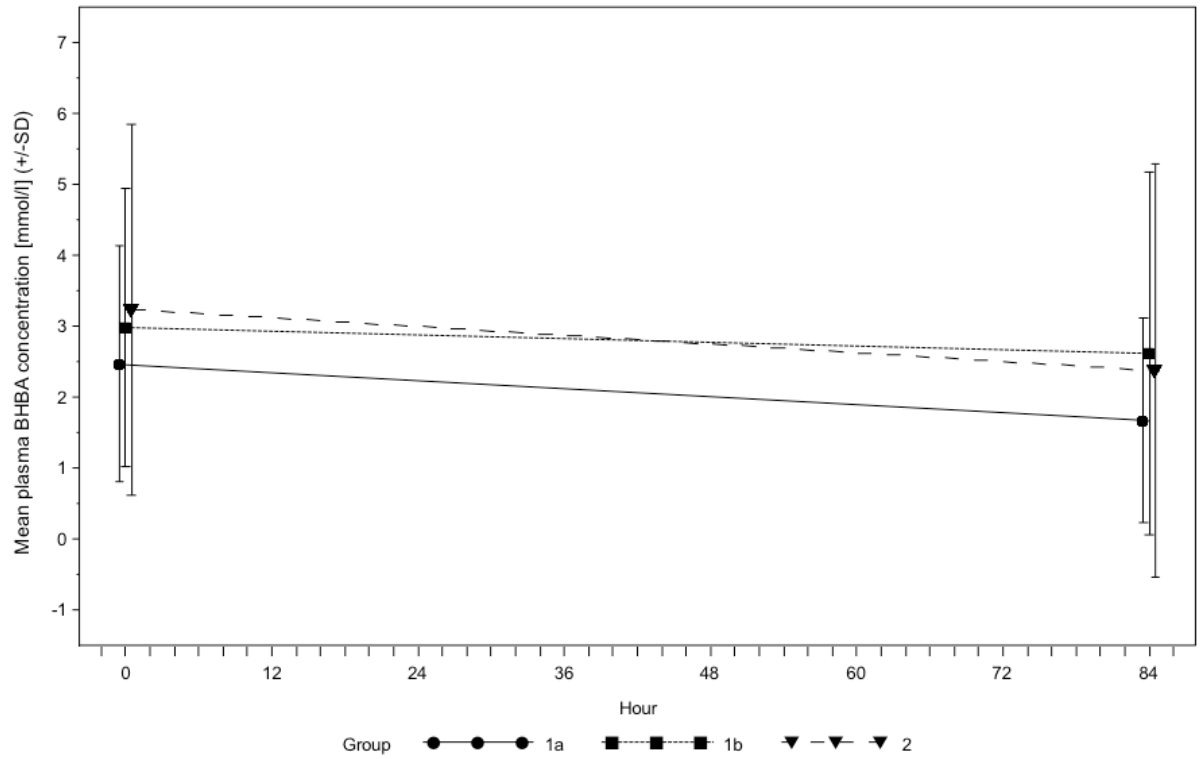

Fig. 14. Time course of mean plasma BHBA concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hrs) and at 84 hrs following bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

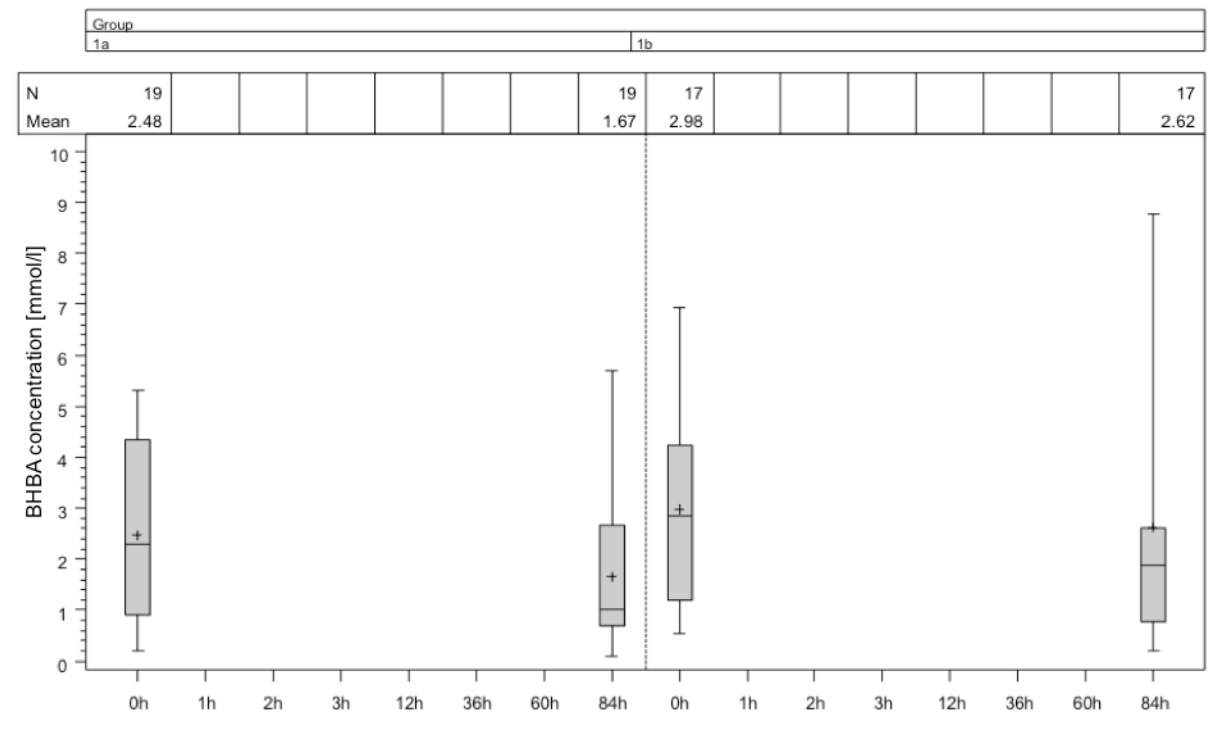

Fig. 15. Boxplots representing plasma BHBA concentrations [mmol/l] in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hrs) and 84 hrs following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.5 Energy Metabolism – Glucose

Plasma glucose levels decreased in animals from all groups following transport and hospitalization and reached the reference range (3.46 – 4.28 mmol/l) as reported by BERTONI and TREVISI (2013) for postpartum cows at 12 hours (Figure 16, Figure 17). Alterations in plasma glucose levels did not show significant differences between groups.

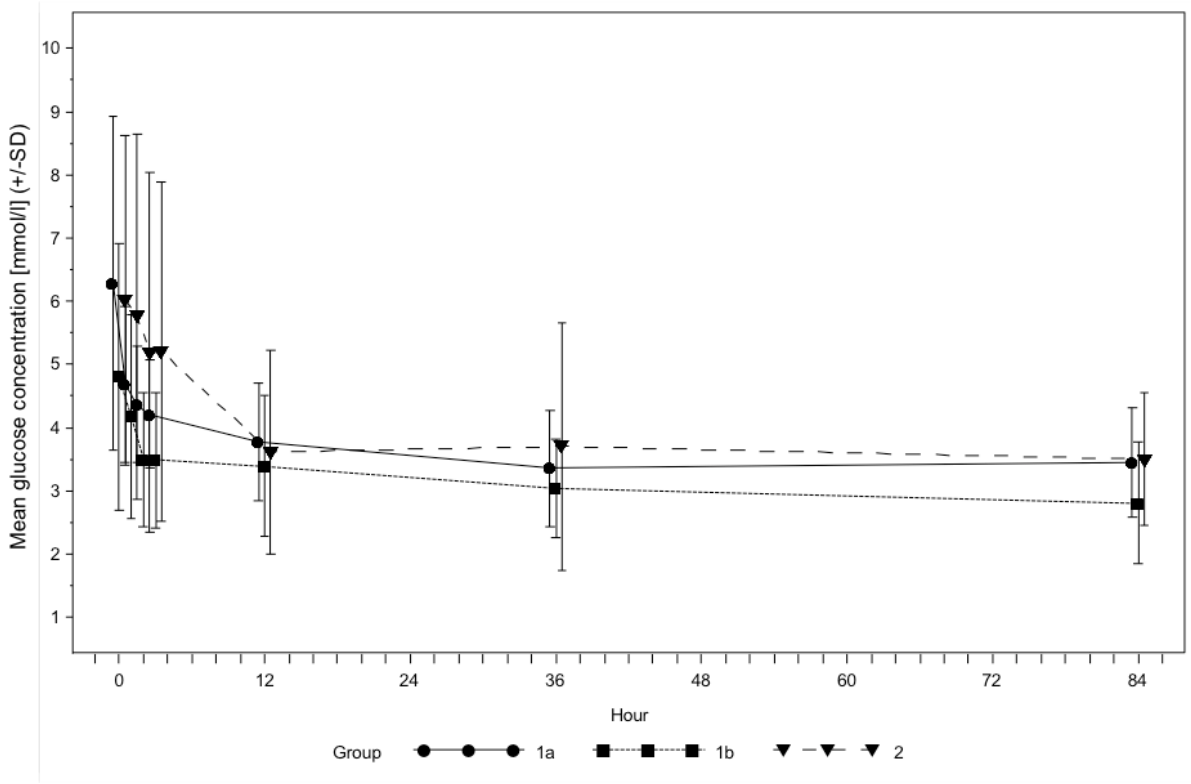

Fig. 16. Time course of mean plasma glucose concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

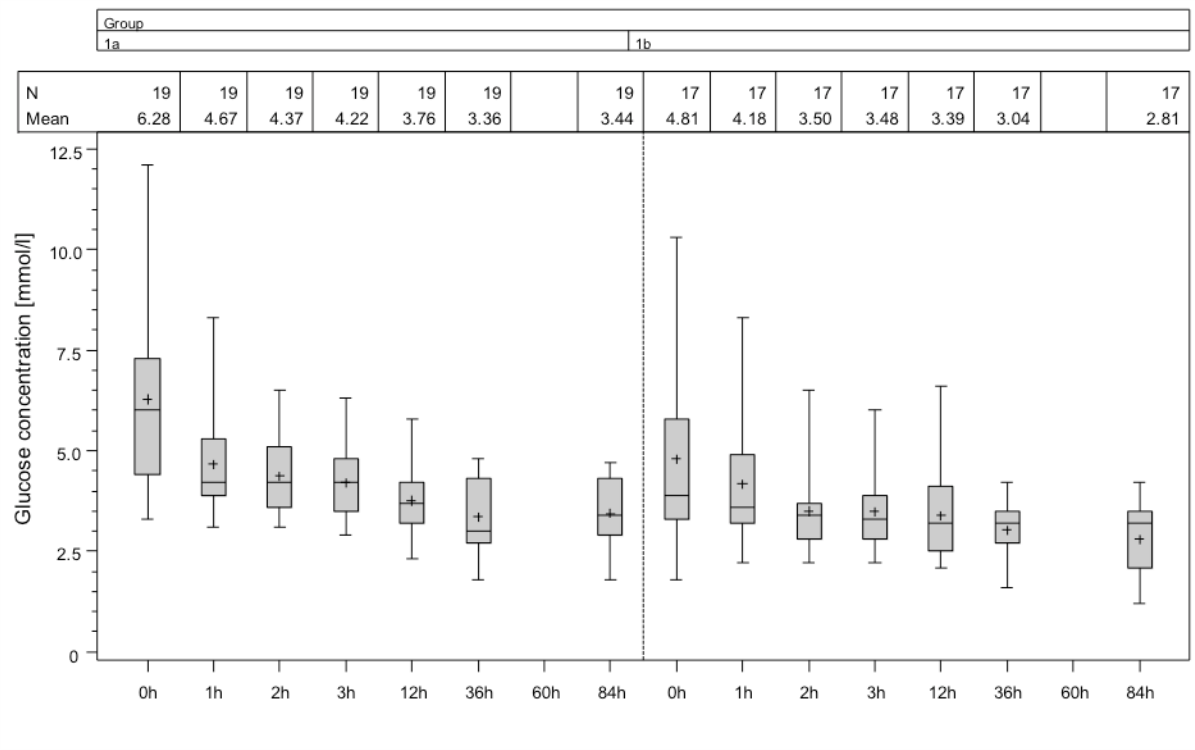

Fig. 17. Boxplots representing plasma glucose concentrations [mmol/l] in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.6 Calcium

Mean plasma concentrations of total calcium (mmol/l) and ionized calcium (Ca^{2+} [mmol/l]) increased in all groups with time but there was no significant group or time effect. Animals from Group 2 had the highest levels of total calcium and ionized calcium over the whole time span of 84 hours.

Animals from all groups showed a decrease in plasma calcium concentrations before surgery but an increase following surgery. At the end of the time span the mean calcium levels of all groups were above the levels at h0 but not significantly higher in any group (Figures 18, 19, 20, 21).

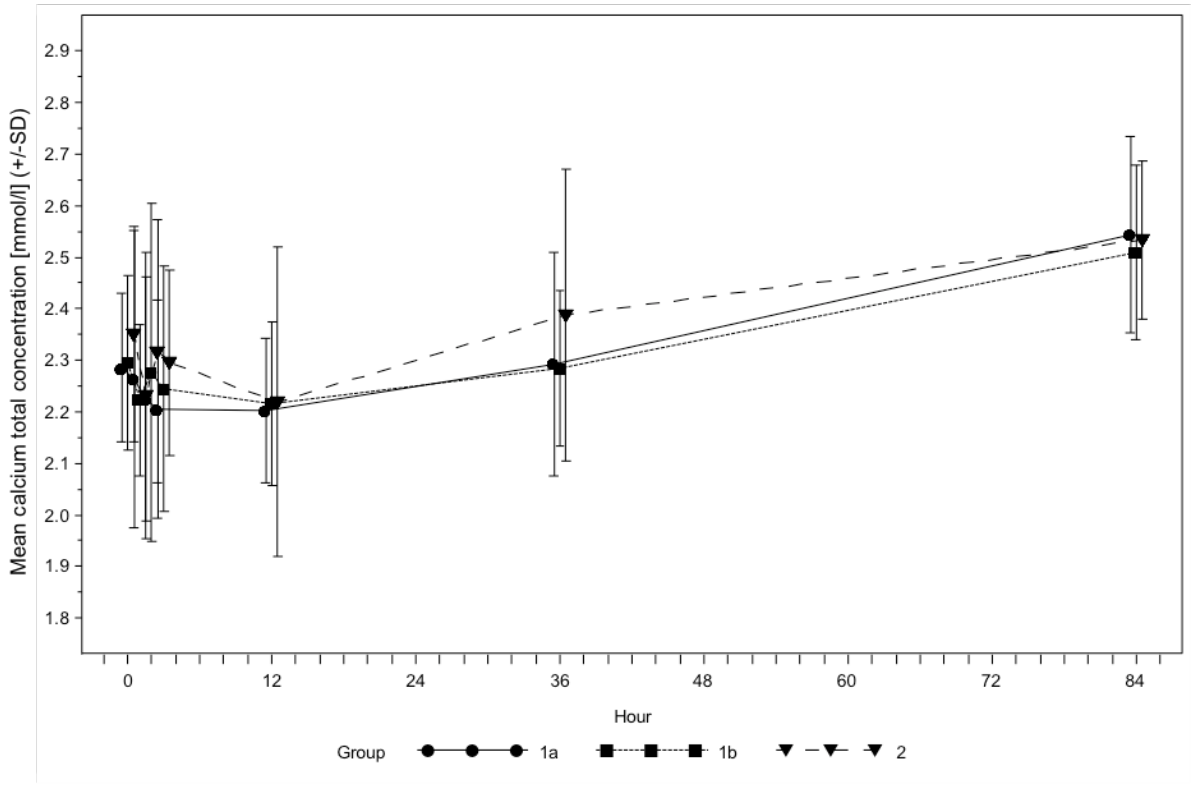

Fig. 18. Time course of mean plasma total calcium concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

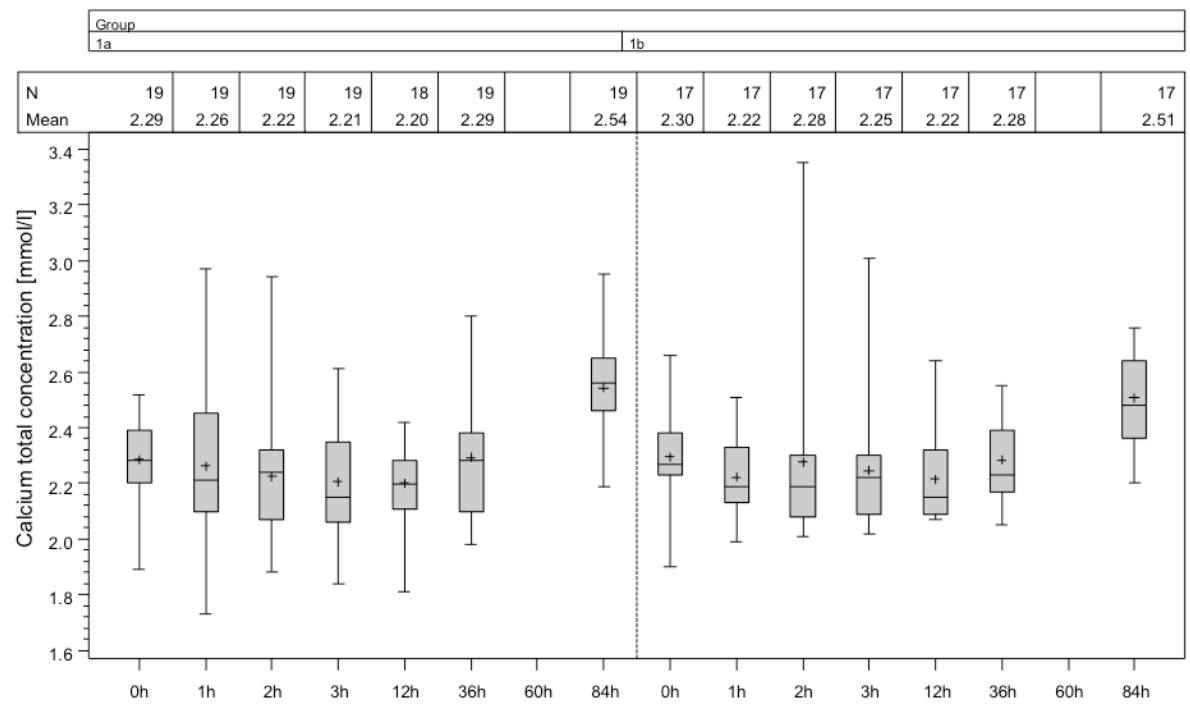

Fig. 19. Boxplots representing plasma total calcium concentrations [mmol/l] in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

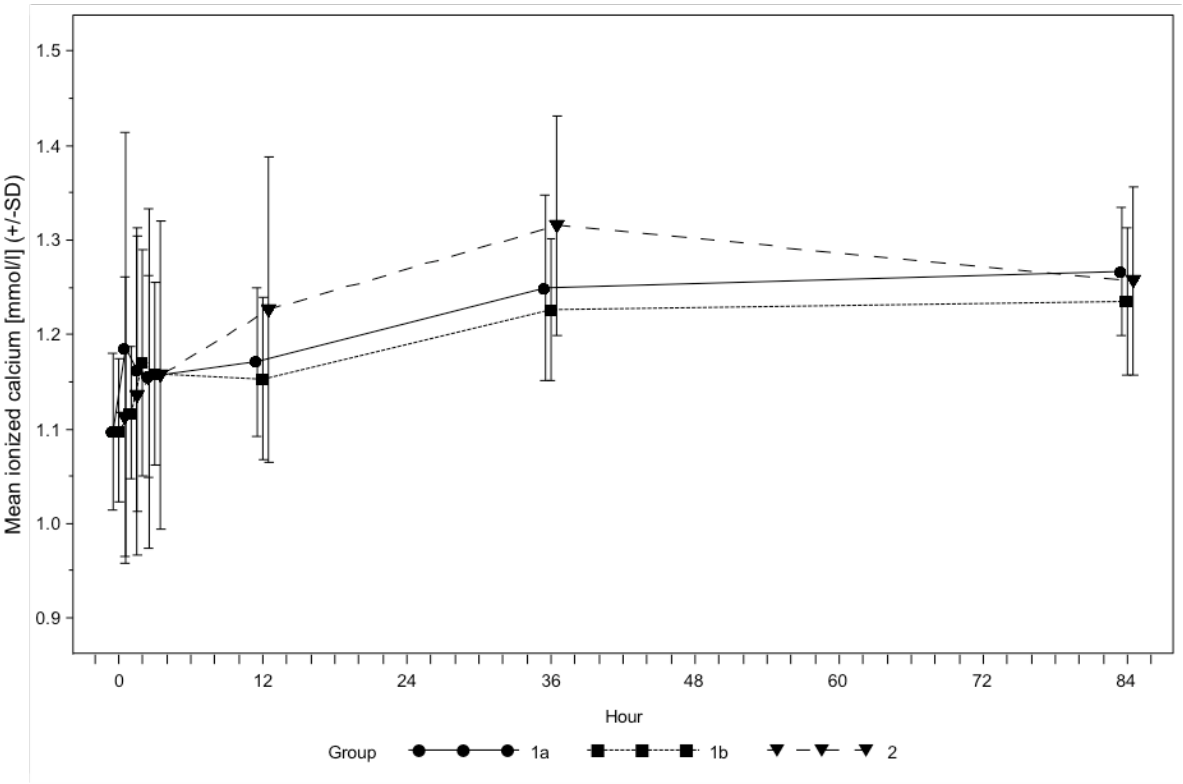

Fig. 20. Time course of mean plasma ionized calcium concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

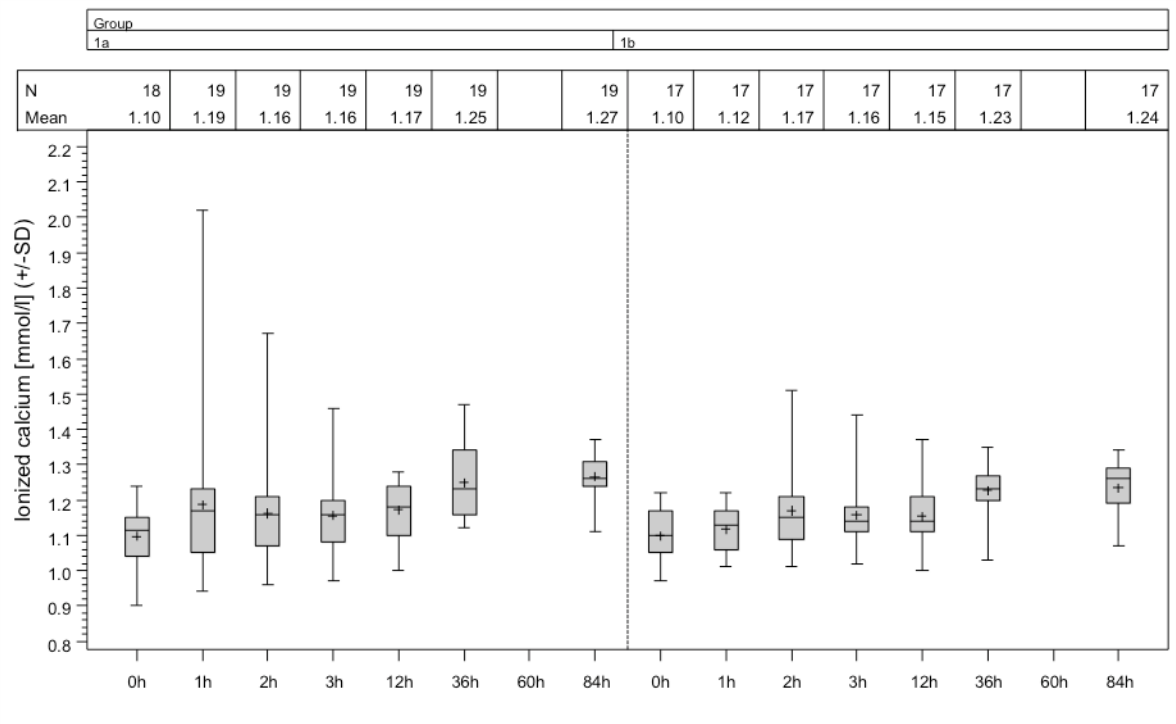

Fig. 21. Boxplots representing plasma ionized calcium concentrations [mmol/l] in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.7 Acid-base-balance

Mean pH, standard bicarbonate (HCO_3^- [mmol/l]) concentrations and Base Excess (BE [mmol/l]) did not show significant differences, neither with time, nor between different treatment groups (Figures 23 – 28).

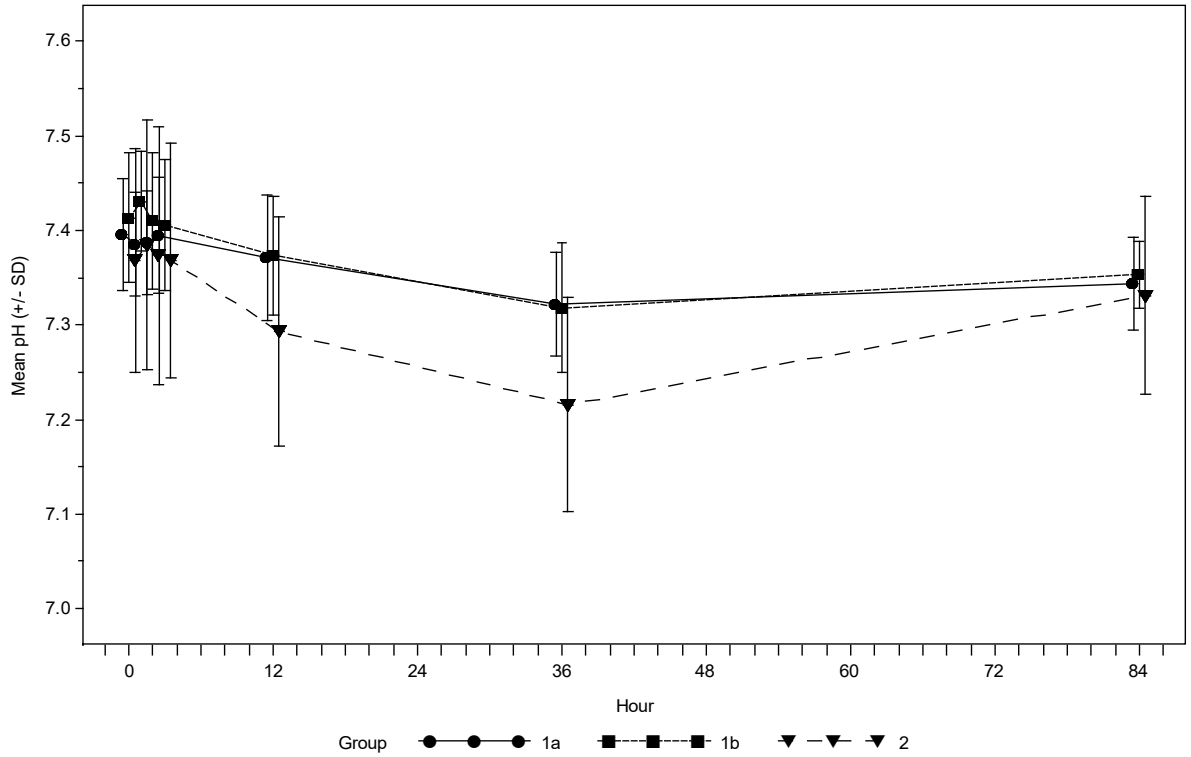

Fig. 22. Time course of mean venous blood pH (± SD) in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

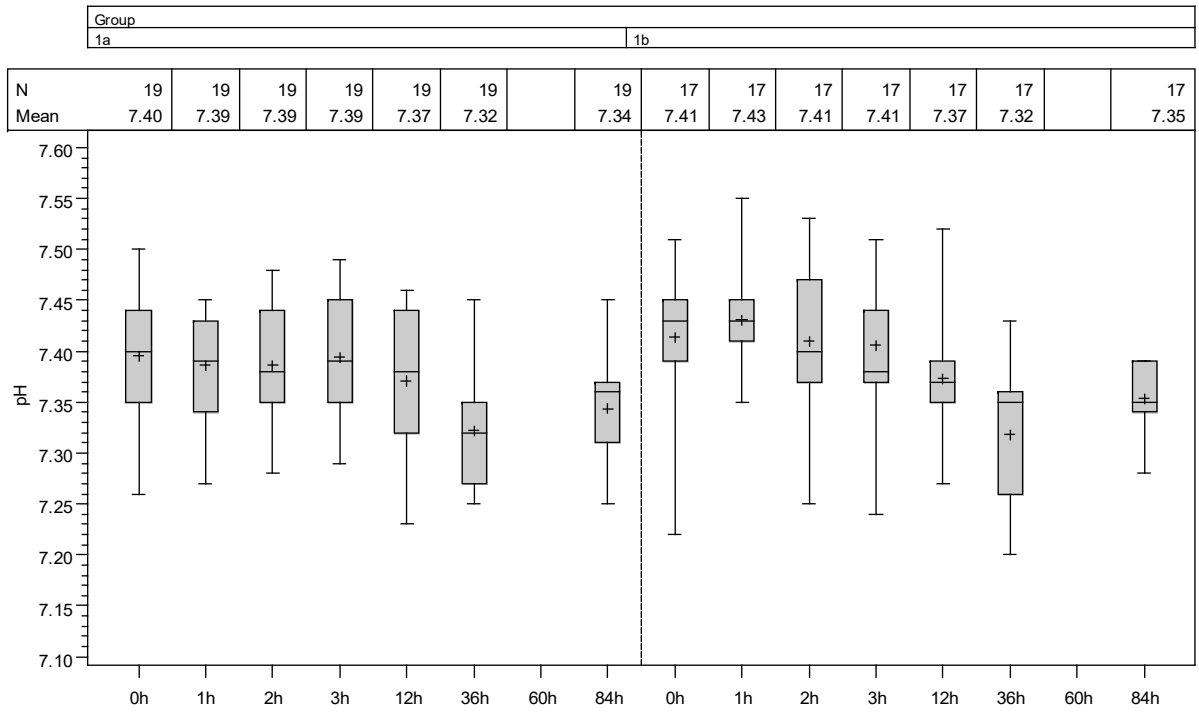

Fig. 23. Boxplots representing venous blood pH in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

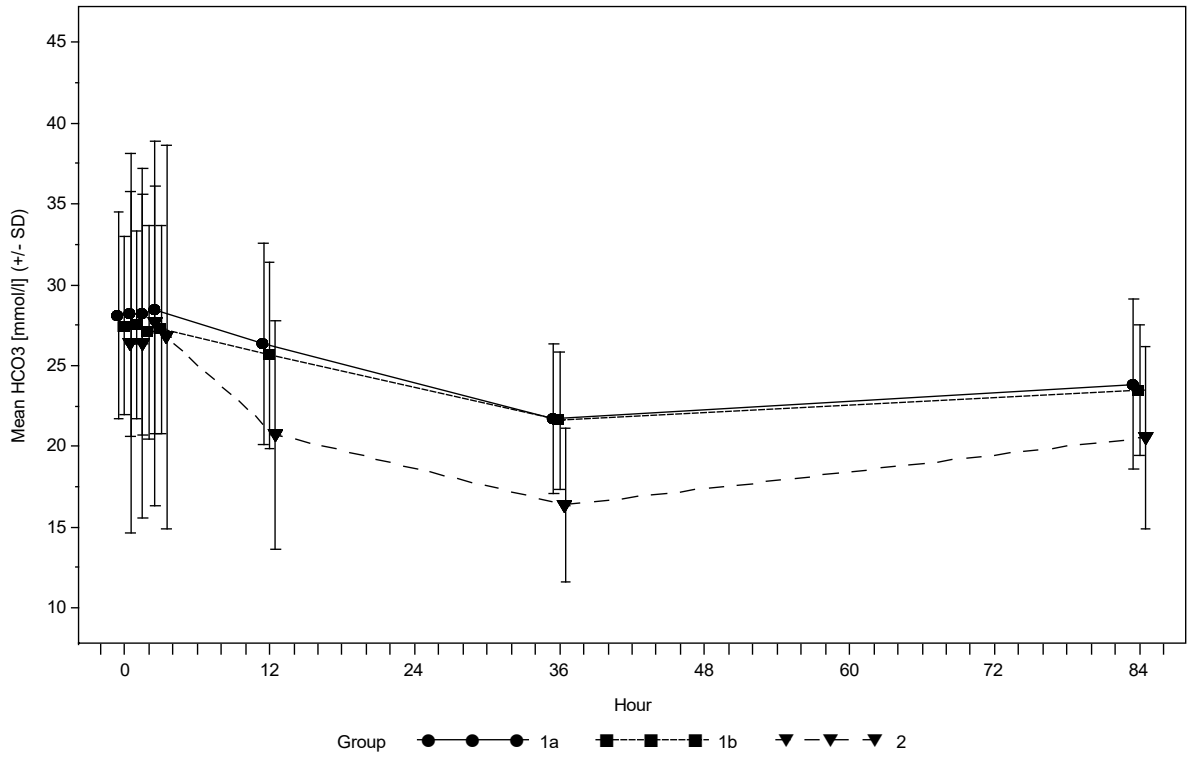

Fig. 24. Time course of mean standard bicarbonate levels [mmol/l ± SD] in venous blood from cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

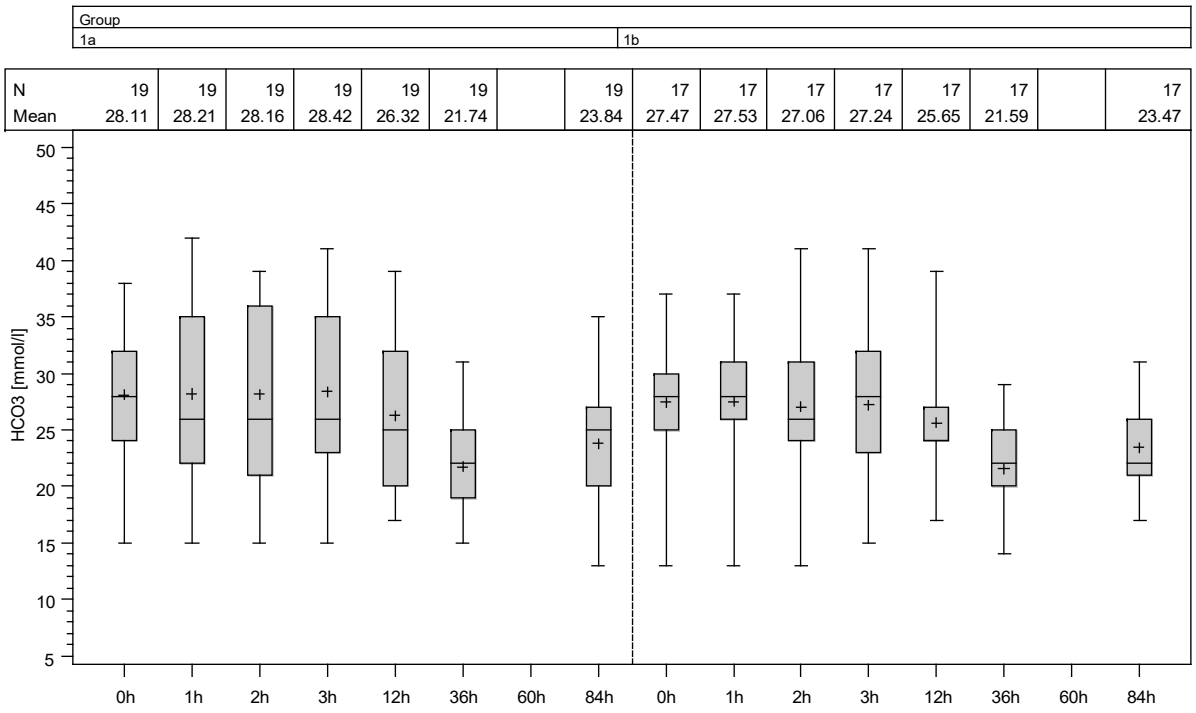

Fig. 25. Boxplots representing standard bicarbonate levels [mmol/l] in venous blood in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

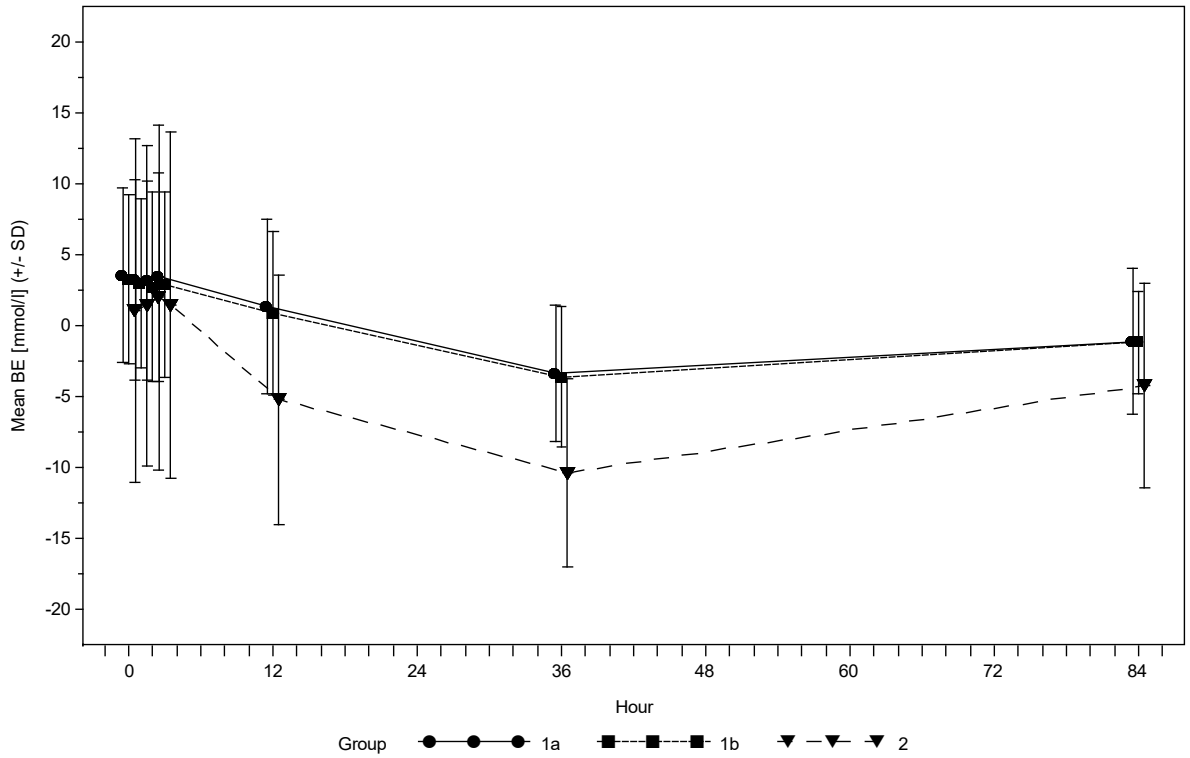

Fig. 26. Time course of mean Base Excess (BE) [mmol/l ± SD] as determined from blood gas analysis of venous blood in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

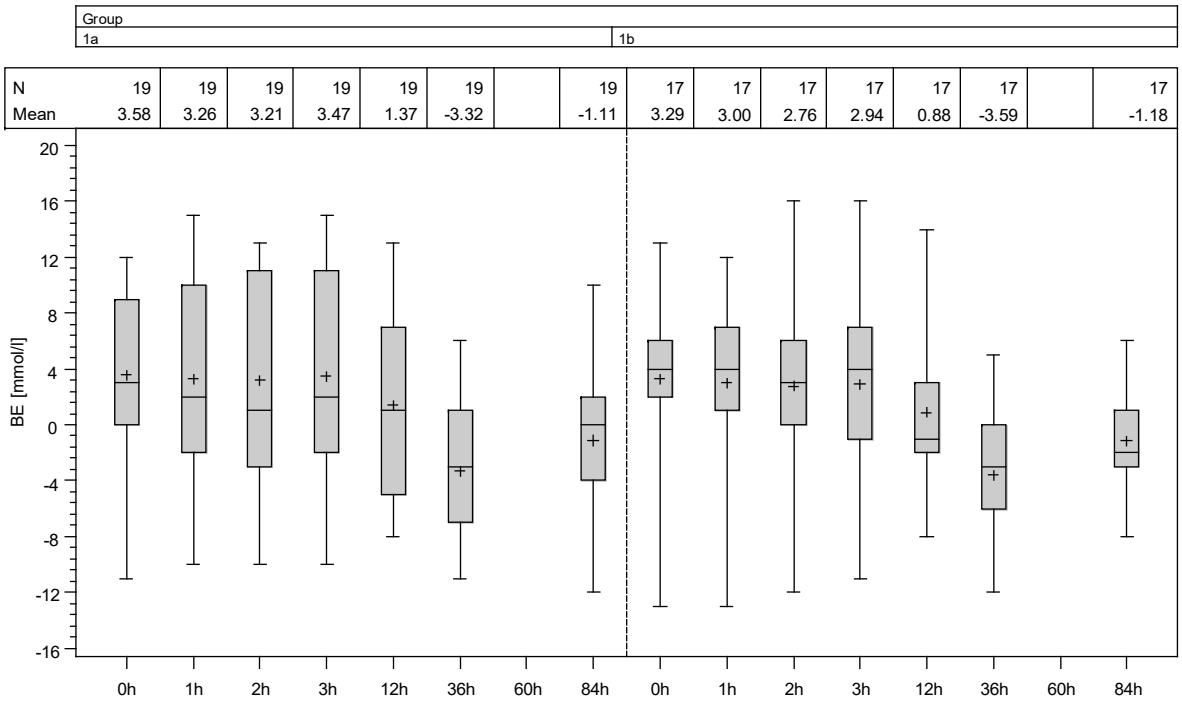

Fig. 27. Boxplots representing Base Excess (BE) [mmol/l] as determined from blood gas analysis of venous blood in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.8 Hemogram

Analysis of hematocrit (HCT [%]), white blood cell counts (WBC [G/l]), red blood cell counts (RBC [T/l]) and platelet counts (PLT [G/l]) did not show significant differences between the groups (Figures 28 – 35).

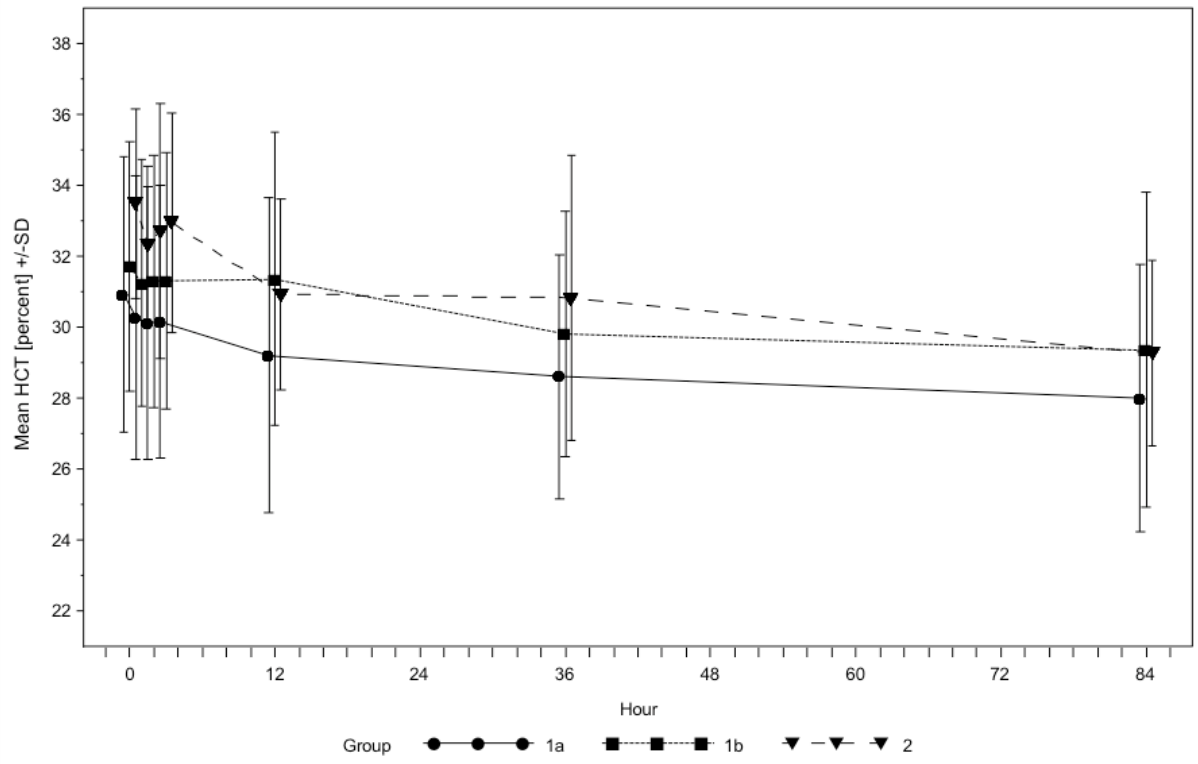

Fig. 28. Time course of mean hematocrit value (HCT [%] ± SD) as determined from venous blood in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

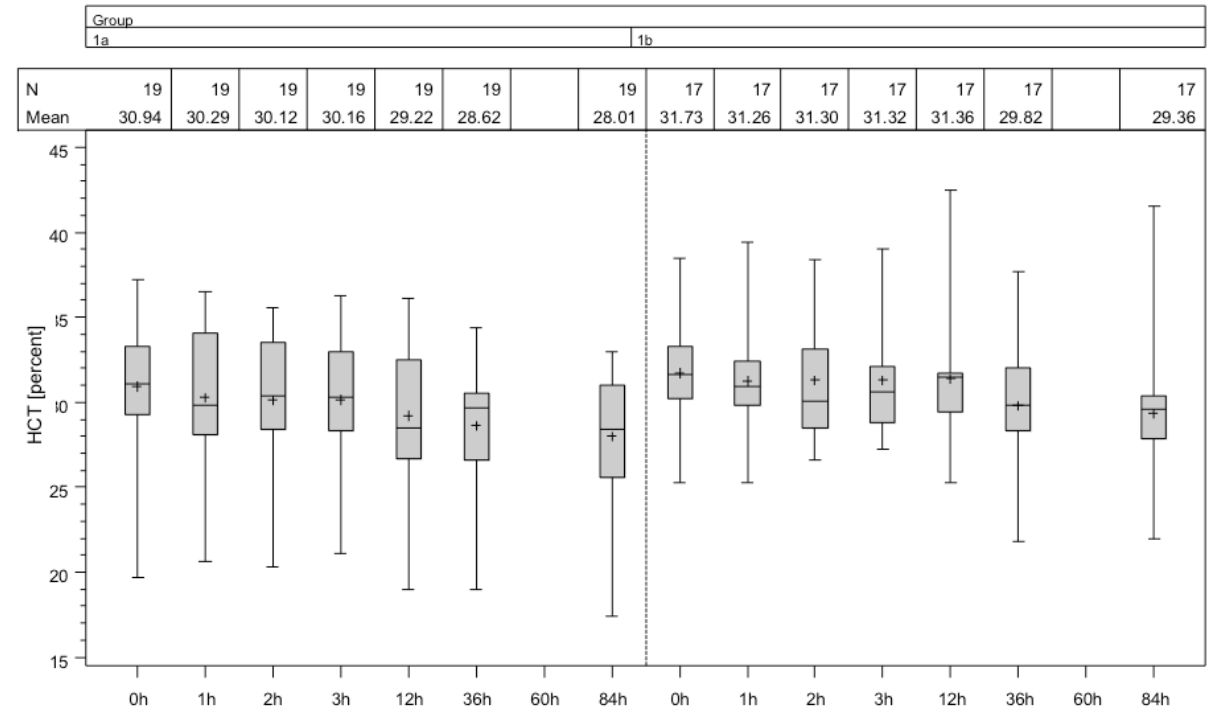

Fig. 29. Boxplots representing hematocrit values (HCT [%]) as determined from venous blood in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

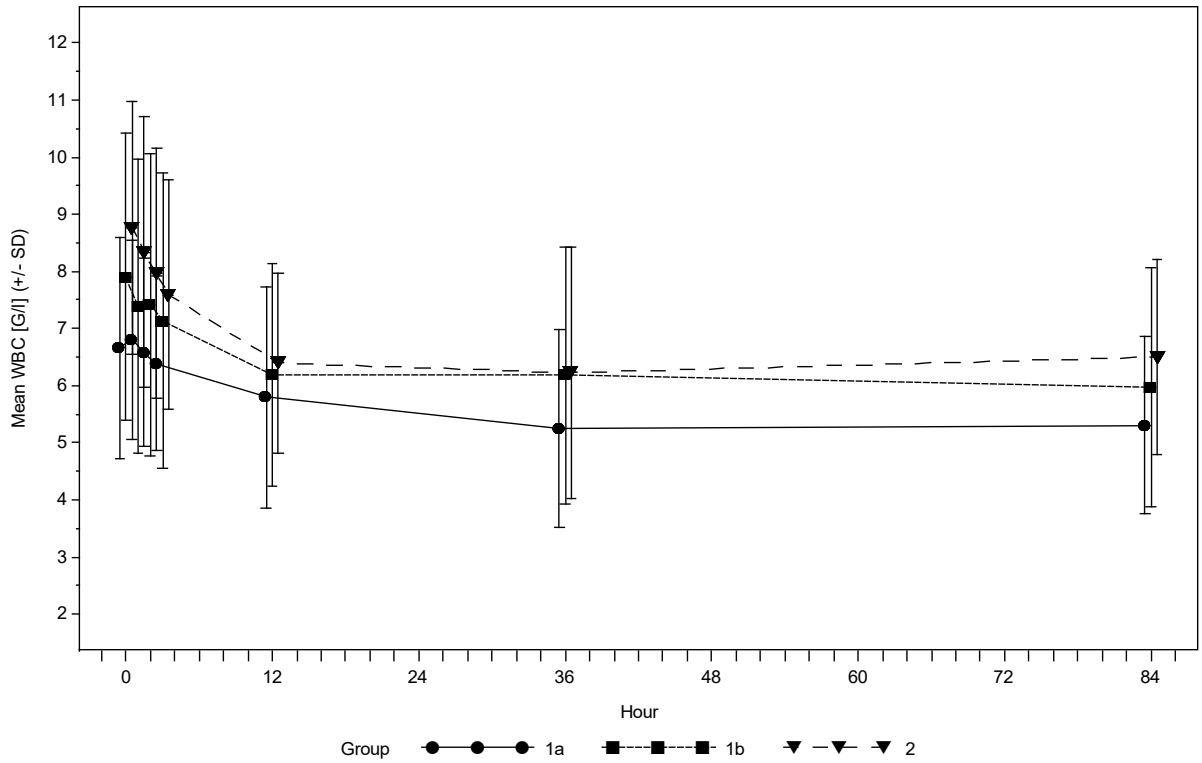

Fig. 30. Time course of mean white blood cell count (WBC [G/l] ± SD) as determined from venous blood in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

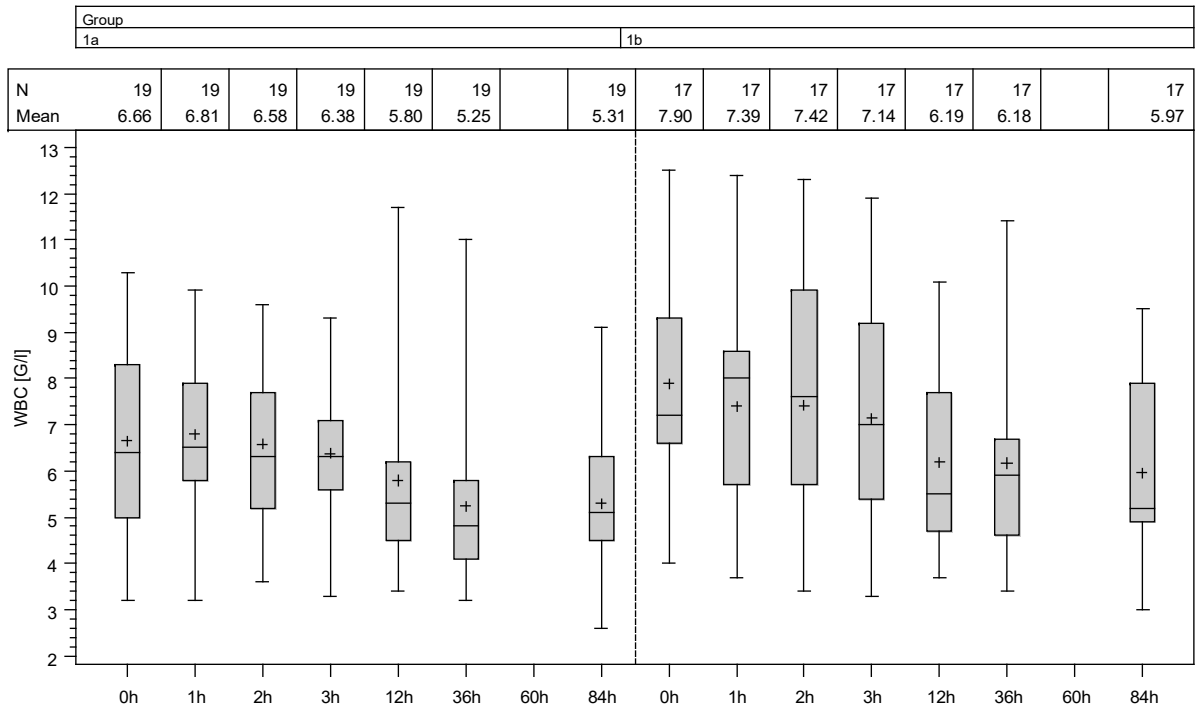

Fig. 31. Boxplots representing white blood cell count (WBC [G/l]) as determined from venous blood in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

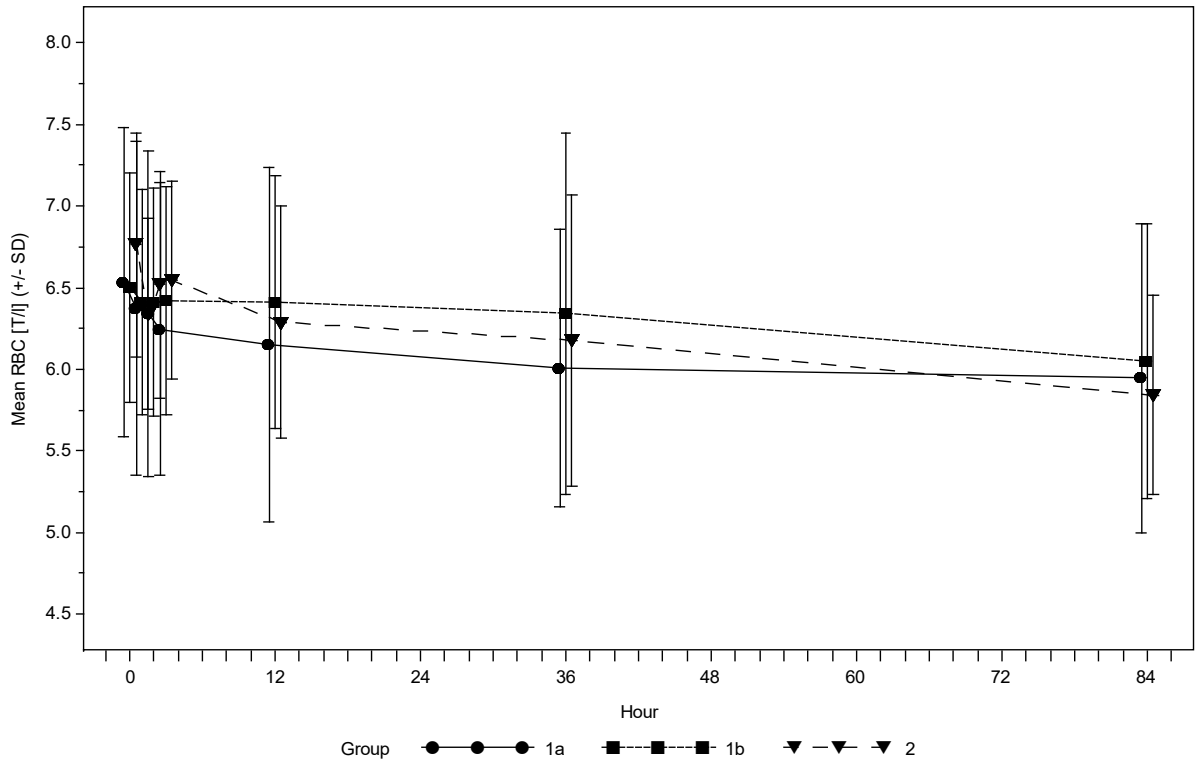

Fig. 32. Time course of mean red blood cell count (RBC [T/l] ± SD) as determined from venous blood in cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

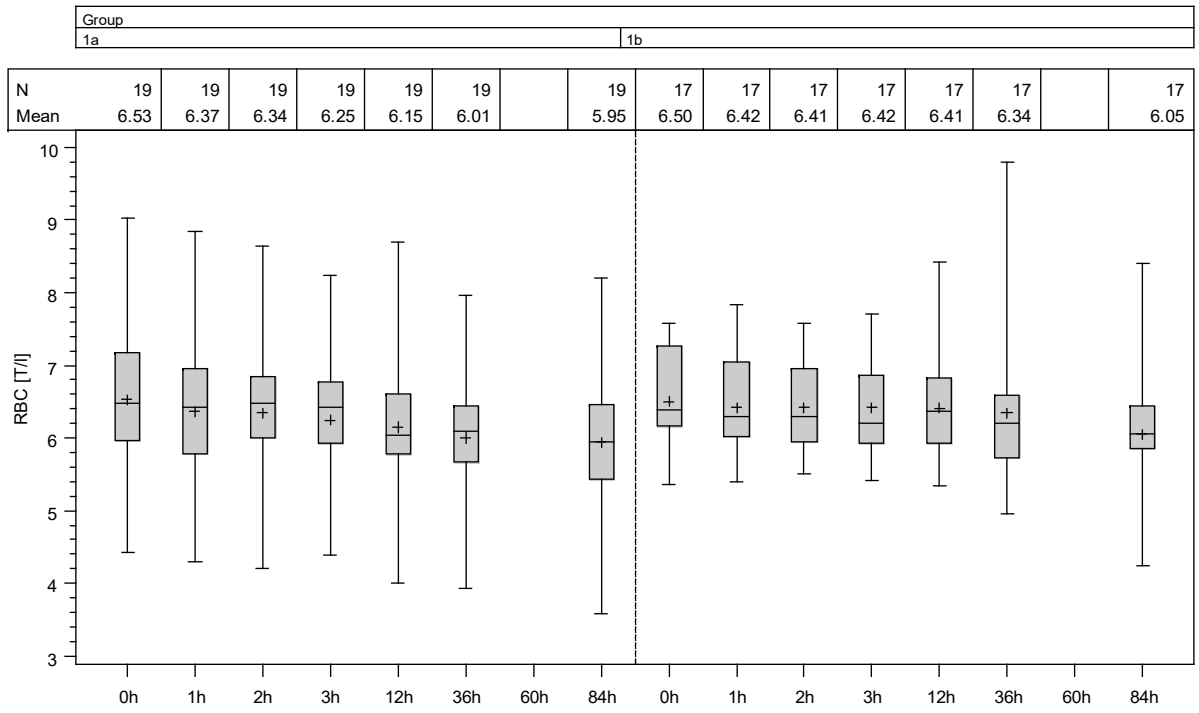

Fig. 33. Boxplots representing red blood cell count (RBC [T/l]) as determined from venous blood in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

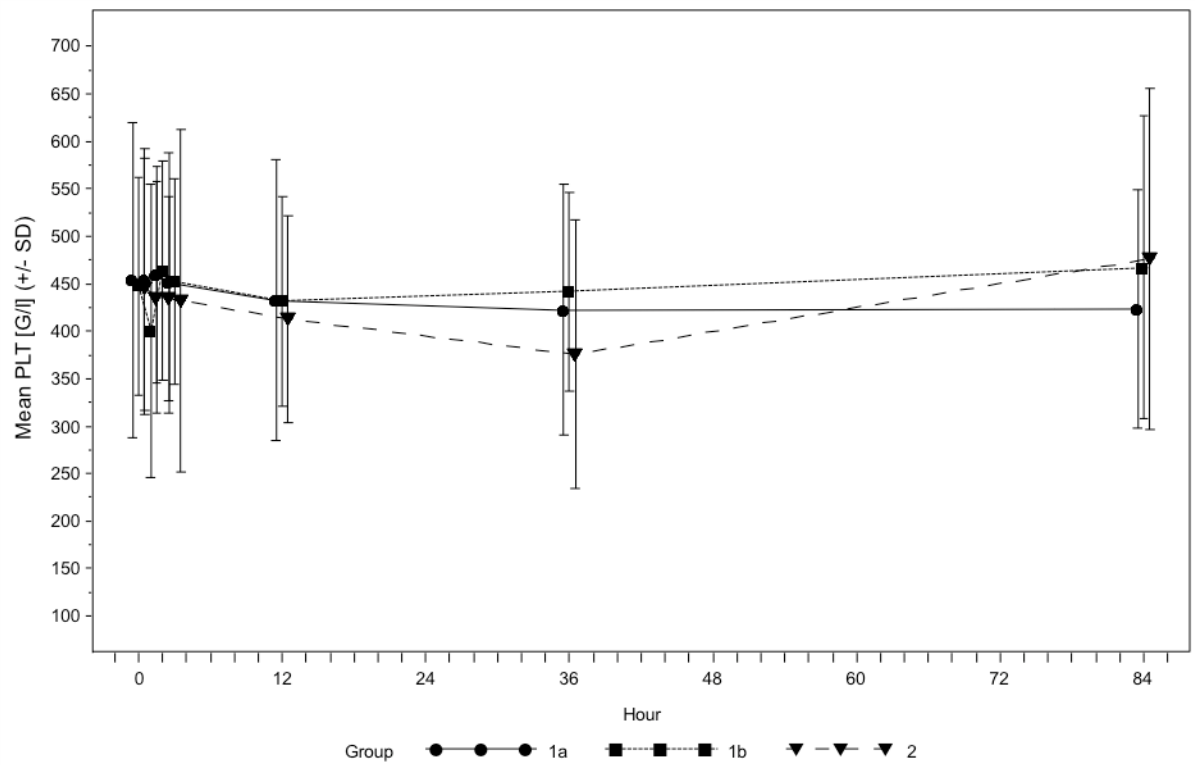

Fig. 34. Time course of mean numbers of platelets (PLT [G/l] ± SD) as determined from venous blood in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

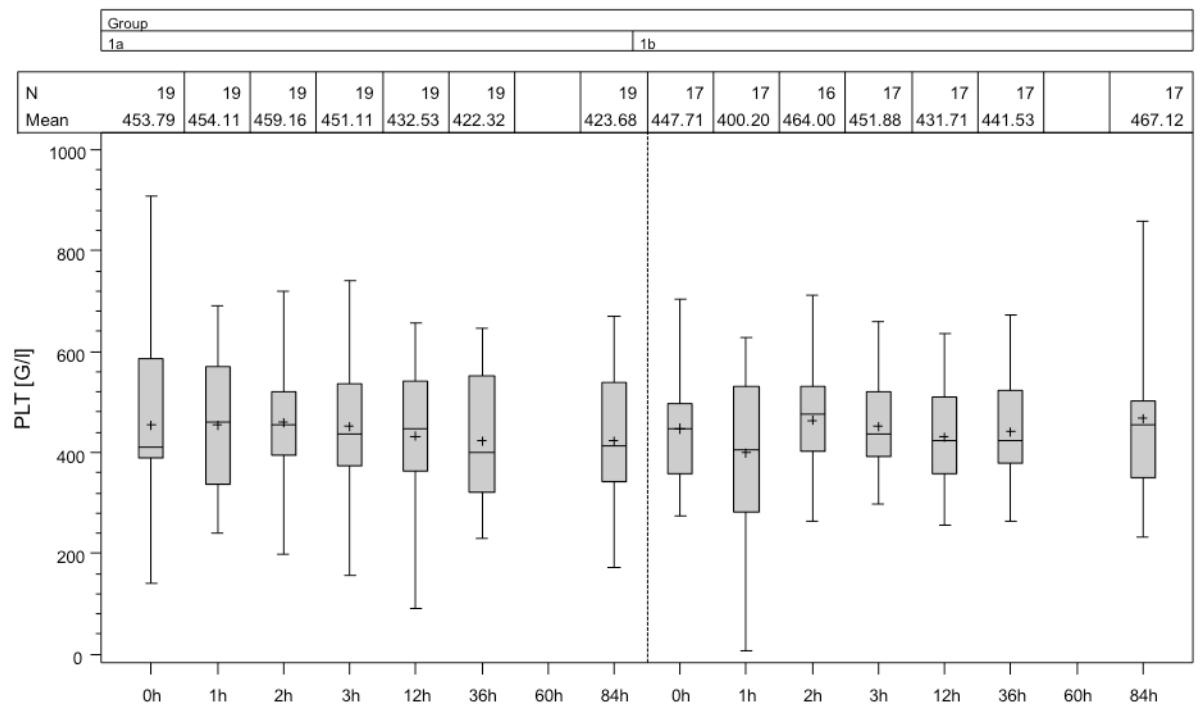

Fig. 35. Boxplots representing numbers of platelets (PLT [G/l]) as determined from venous blood in two groups of cows with hypokalemia. Results are presented for blood samplings before (0 hours) and at different time points following potassium bolus administration (Group 1a) and sham administration (Group 1b). The ends of the whiskers represent minimum and maximum.

5.1.8.9 Sodium, Chloride, Lactate, Creatinine, BUN, Alb, bilirubin, AST, gamma-GT, AP, Phosphate, Total Magnesium

There were no significant changes observed for the following variables neither with time, nor between different treatment groups (Fig. 36 – Fig. 47, see Appendix):

plasma sodium, chloride, lactate, creatinine, blood urea nitrogen (BUN), albumin (Alb), bilirubin, phosphate and total magnesium concentrations, activities of aspartate amino-transferase (AST), gamma-glutamyltransferase (gamma-GT), alkaline phosphatase (AP), phosphate and total magnesium concentrations.

5.2 Follow-up Data

Follow-up information was collected via client questionnaires. A standardized questionnaire gathered the following information: if the animal was still on farm, the outcome on farm (died, slaughtered, euthanized, sold, still on farm, still on farm and inseminated, still on farm and pregnant), insemination date if inseminated, date of positive pregnancy examination if pregnant, exit date if not on farm anymore, milk kg per day before admission, milk kg per day after admission. Depending on the milk yield, cows were assigned to three different categories A > 30 kg per day, B 10 – 30 kg per day and C < 10 kg per day.

Follow-up information was available for 37 from 47 cows (78.7%) and was retrieved in a time span between September 2013 (35) and July 2014 (2). Table 12 shows the number of animals per group from which follow-up data were available and the response rate for each group separately.

Assuming that cows that either display problems during their convalescence, or that do not fulfill the expectations of the owner with respect to getting back into lactation would have left the farm within one month following their dismissal from the clinic, the remain of the cows was evaluated for those animals that remained less than one month following the dismissal on the farm and those that remained on the farm for more than one month and beyond following their dismissal from the clinic. 86.7 (13) and 92.9% (13) of cows from Group 1a and 1b, respectively, were still on the farm at one month and beyond following the dismissal from the clinic. This was true for six out of a group of 8 cows that belonged to Group 2 (Table 13).

Table 12. Follow-up data. Number of returned data sets and response rates presented separately for each treatment group.

	Follow-up data received		
	yes	no	%
Group			
1a	15	4	78.9
1b	14	3	82.4
2	8	3	72.7

Table 13. Remain time on farm of cows with hypokalemia after dismissal from the animal hospital. Categories: still on farm at < 1 month after dismissal, still on farm at > 1 month after dismissal; Data are given as total numbers of retrieved records per group as well as total numbers and percentage from retrieved records for each category.

	Remain time on farm*			
	< 1 month after dismissal		> 1 month after dismissal	
Group	N	%	N	%
1a	2	13.3	13	86.7
1b	1	7.1	13	92.9
2	2	25	6	75

*as far as information was given

As displayed in Table 13, a majority of cows were still on their farms of origin one month after completion of the study. As far as information was provided, the average number of days animals spent on their farms before being sold, slaughtered, euthanized or deceased was 74 for Group 1a (N = 5), 129 for control Group 1b (N = 3) and 6 days for Group 2 (N = 1). At the day of the inquiry one animal of Group 1a and Group 1b, respectively, and two animals of Group 2 had died or had been euthanized and four (26.7 %) animals from Group 1a and two (14.3 %) animals from Group 1b were slaughtered or sold while 10 (66.7 %), 11 (78.6 %) and 6 animals in the respective Groups 1a, 1b and 2 were still on the farm (Table 14).

Table 14. Fate of hypokalemic cows after dismissal from the animal hospital. Data are given as total numbers as well as percentage of retrieved follow-up data in each group.

	died or euthanasia		slaughtered or sold		still on farm	
Group	N	%	N	%	N	%
1a	1	7.7	4	26.7	10	66.7
1b	1	7.1	2	14.3	11	78.6
2	2	25	0	0	6	75

Table 15 presents the results with respect to **reproduction data** of cows following their dismissal. The low number of animals that were 50 days post partum and beyond (period to first insemination) or for which pregnancy was assured does not allow to draw any conclusions with respect to the different treatment groups.

Table 15. Day of insemination and pregnancy of cows with hypokalemia after dismissal from the animal clinic. Data are given as total numbers of retrieved records as well as percentage from retrieved records.

			inseminated at						confirmed pregnancy			
	not yet inseminated*		< 50 days p.p.		50-100 days p.p.		> 100 days p.p.		yes		no	
Group	N	%	N	%	N	%	N	%	N	%	N	%
1a	6	40	0	0	5	33.3	4	26.7	2	13.3	13	86.7
1b	4	28.6	1	7.1	2	14.3	7	50	2	14.3	12	85.7
2	5	62.5	0	0	1	12.5	1	12.5	1	12.5	6	75

*or no information given

As mentioned above, cows were assorted to **milk yield categories** A, B and C (Table 16, Table 17). None of the cows from which follow-up data were available switched to the category of a milk yield below 10 liters (C) after being released from the animal hospital. Four animals from Group 1a and two animals from Group 1b, respectively, turned from a milk yield of 10-30 liters (B) before hospitalization to a milk yield of more than 30 liters after hospitalization (A). One animal belonging to Group 1a and one animal belonging to Group 1b showed a decrease in milk yield (A → B), both were still on farm at 353 (1a) and 121 (1b) days after completion of the study (Table 18). One cow of Group 2 showed an increase in milk yield of two categories (C → A). Missing data was due to missing information by the local herd managers, as well as animals that did not make a full recovery after completion of the study and therefore left the herd shortly after return to their farms of origin. One cow of Group 1a was not lactating at admission to the study due to pregnancy and was euthanized 40 days after return to the herd.

Milk yield categories did not change significantly in either of the study groups. 33.3 % of the cows belonging to Group 1a and 21.4 % of the cows belonging to Group 1b changed from one category to another, whereas one animal of each group showed a decrease in milk yield (Table 18).

Table 16. Total numbers and percentage of animals of different treatment groups and control group assorted to milk yield categories before hospitalization. Milk yield categories A > 30 kg per day, B 10 – 30 kg per day and C < 10 kg per day; Missing = missing data.

	Milk Yield Category before hospitalization									
	Missing		A		B		C		All	
	N	%	N	%	N	%	N	%	N	%
Group										
1a	5	33.3	3	20	7	46.7	0	0	15	100
1b	5	35.7	3	21.4	6	42.9	0	0	14	100
2	1	12.5	0	0	6	75	1	12.5	8	100

Table 17. Total numbers and percentage of animals of different treatment groups and control group assorted to milk yield categories after hospitalization. Milk yield categories A > 30 kg per day, B 10 – 30 kg per day and C < 10 kg per day; Missing = missing data.

	Milk Yield Category after hospitalization									
	Missing		A		B		C		All	
	N	%	N	%	N	%	N	%	N	%
Group										
1a	5	33.3	6	20	4	46.7	0	0	15	100
1b	4	28.6	3	21.4	7	50	0	0	14	100
2	3	37.5	3	37.5	2	25	0	0	8	100

Table 18. Total numbers and percentage of changes in milk yield categories of study animals. Milk yield categories A > 30 kg per day, B 10 – 30 kg per day and C < 10 kg per day; Missing = missing data.

	Number of Milk Yield Categories Change									
	Missing		0		1		2		All	
	N	%	N	%	N	%	N	%	N	%
Group										
1a	5	33.3	5	33.3	5	33.3	0	0	15	100
1b	6	42.9	5	35.7	3	21.4	0	0	14	100
2	3	37.5	2	25	2	25	1	12.5	8	100

5.3 Treatment failures

One of the study animals (B120293, Group 2, 3 boluses) had to be euthanized on day three of the study due to persistent acidosis and fatty liver leading to liver coma and terminal recumbency. B130028 (Group 1b, no bolus) showed a plasma potassium concentration of 3.2 mmol/l on day 5 and was euthanized after completion of the study due to peritonitis and persisting ketosis and metritis. B130043 (Group 1b, no bolus) underwent successful surgery but was euthanized after 10 days of hospitalization suffering from a severe phlegmonous inflammation of the distal limb. B130134 (Group 2, 2 boluses) received rescue treatment of dexamethasone on day three due to persistent ketosis but was nevertheless euthanized on day 5 due to abomasitis geosedimentosa in combination with rhabdomyolysis and fatty liver, which implicated unfavorable prognosis. B130209 (Group 2, 3 boluses) was presented with

ketosis and fatty liver. BHBA concentrations did not improve over time despite rescue treatment (dexamethasone) on day three and plasma potassium concentrations stayed below 3.5 mmol/l. Also, muscle potassium concentrations on day 5 showed depletion of intracellular reservoirs (day 0: 3.3 mg/kg; day 5: 2.4 mg/kg). B130019 (Group 1a, 2 boluses) was presented with left abomasal displacement. In surgery, an abomasal ulcer and chronic peritonitis were detected, after which the animal developed vagus indigestion. The cow completed the study with no adverse events and was euthanized thereafter due to unfavorable prognosis of the Hoflund Syndrome.

6 DISCUSSION

6.1 Background and hypothesis

Potassium is the third most abundant mineral of the body. Potassium from the diet is absorbed in the gastrointestinal tract and those amounts that exceed the requirements of dairy cattle are excreted via the kidney in a very efficient manner (NATIONAL RESEARCH COUNCIL, 2001; PALMER, 2015). Anorexia has been shown to cause moderate hypokalemia (RABINOWITZ et al., 1988). In case of reduced potassium uptake due to starvation, or due to sequestration of K^+ in the rumen in case of abomasal reflux syndrome, the body potassium pool can to a certain extent be maintained by decreasing renal excretion of potassium. In healthy animals, following feed uptake, potassium is transported into the body cells by an insulin-dependent mechanism that activates the energy dependent Na^+/K^+-ATP-ase. To this end, starvation reduces potassium movement from the extracellular to the intracellular space due to hypoglycemia. Due to the latter mechanisms extracellular potassium levels as reflected in serum potassium concentration decrease only gradually in healthy animals and man following starvation (PALMER, 2015). Periparturient dairy cows demonstrating inappetence or even anorexia have been shown to lose potassium due to high milk yield and – in summer – due to heat stress. To this end, the latter cows experience a negative potassium balance. Various pathological conditions (lipomobilization and ketosis, abomasal displacement, abomasal reflux syndrome, septic mastitis or metritis) (SATTLER et al., 1998; PEEK et al., 2000), however, have a detrimental effect on potassium homeostasis by a combination of risk factors for hypokalemia caused by indirect effects on various transporters that are related with potassium secretion in the kidney and internal potassium transport (PALMER, 2015). When the body is not able to compensate for said negative potassium balance, hypokalemia develops (GOFF, 2006; HARRISON et al., 2011). The importance of potassium for the maintenance of acid-base-homoeostasis, membrane potential and neuromuscular transmission as well as the consequences of hypokalemia have intensively been studied and to this end, the postpartum high yielding dairy cows displaying anorexia for several reasons are a target group for potassium supplementation. Administration of potassium via oral route has been shown to be the most efficient and safe way to restore potassium levels in hypokalemic cows (GREENLEE et al., 2009; CONSTABLE et. al. 2014).

The present study examined the hypothesis, that the administration of a bolus containing potassium to hospitalized hypokalemic dairy cows suffering from various production diseases is not only a safe and convenient method to restore plasma potassium levels, but does also have a beneficial influence on appetite, milk yield, ruminal activity, energy metabolism, position of the abomasum and general convalescence of affected animals.

6.2 Study design

Inclusion criteria for the present clinical trial were preset on basis of the characteristics of the potential target group for oral potassium supplementation. Hypokalemic lactating dairy cows, that suffered from LDA, ketosis, metritis and mastitis as single entities or in combination were eligible. Patients with potassium concentrations of ≥ 2.8 and < 3.5 mmol/l (moderate hypokalemia) were assorted to treatment Group 1a or control Group 1b, respectively, whereas all study subjects with a plasma potassium concentration of < 2.8 mmol/l (severe hypokalemia) were assorted to treatment Group 2. Assignment to the groups was performed after first blood analysis at h0. The patients were referred to the clinic by local practitioners, which required close collaboration and compliance of the clinic, the practitioners, farm owners and herd managers, respectively. Hence it has to be taken into account, that patients

admitted to an animal hospital belong to a preselected population, of which some of the animals had already received a treatment which failed. It may furthermore be presumed that animals, which would theoretically have been eligible for the trial (e.g. cows with moderate hypokalemia and mastitis or metritis) were not always referred to the clinic but treated on farm. Precise and rapid analysis of potassium values and other blood parameters as well as the proper observation and examination of study animals (especially concerning feed intake and milk yield) required patient hospitalization; however, with the improvement of cow-side tests for various blood parameters (ROLLIN, 2006; ABUELO and ALVES-NORES, 2016) the possibility of a similar trial setting under field conditions should be considered for the future in order to include a larger number of candidates for the evaluation of the influence of potassium on whole body functions *in vivo*.

6.3 Clinical findings in hypokalemic cows

In total, 47 high yielding dairy cows hospitalized in the clinic with hypokalemia were included in the clinical trial. 37 (78.7 %) of these animals suffered from LDA, 32 (68.1 %) from ketosis, 2 (4.2 %) from mastitis and 16 (34 %) from metritis as single entities or in combination. There was no significant difference concerning days in milk between the different groups. Animals belonging to Group 1a or 1b, i.e. suffering from moderate hypokalemia, were averagely 15.2 (2-43) days post partum, whereas cows with severe hypokalemia (Group 2) had calved on average 18.1 (11-33) days before admission to the clinic. It is worthy of note that cows solely suffering from ketosis were on average 21.5 (8-43) days in milk. However, as in the present clinical trial data from hypokalemic animals with various concomitant diseases was collected, it proved difficult to evaluate the influence of a single disorder on potassium homeostasis and patient outcome. The findings of the present trial are consistent with those in scientific literature, as hypokalemia mostly occurred in the transition period and was commonly associated with diseases that entailed a decreased dietary intake and various pathological conditions. Hypochloremic, hypokalemic metabolic alkalosis was reported for several patients suffering from LDA in literature (KUIPER and BREUKINK, 1980; HALPERIN and KAMEL, 1998; DIRKSEN, 2006 b.; KALAITZAKIS et al, 2010; CONSTABLE et al., 2013). In these cows, surgical correction in most cases resulted in a reconstitution of serum potassium levels, while surgery and potassium supplementation gave a much faster effect with respect to return of the serum potassium levels to the reference range. However, hypokalemia in combination with (keto-) acidosis and fatty liver proved to be associated with a worse general condition and, in severe cases, unfavorable prognosis, which corroborates the findings of FUBINI and DIVERS (2007) and KALAITZAKIS et al. (2010).

The consequences of severe hypokalemia were clearly demonstrated by study animals suffering from plasma potassium concentrations < 2.8 mmol/l (Group 2). Those animals often showed weakness especially of the muscles in the neck, which resulted in difficulties of carrying the weight of the own head. The affected cows would often be found carrying their heads low and sometimes resting their heads on the trough or lying with their heads resting on the lateral side of their body (auto-auscultation). Muscle fasciculations of the *anconeus muscle group* were observed in some of the study animals, while these struggled to maintain a standing position. Signs of lassitude and fatigue could be observed in almost all of the animals at the beginning of the study. This supports the findings of PEEK et al. (2000), who described 17 cows with severe hypokalemia, 10 of which had to be euthanized due to recumbency and unfavorable prognosis. Our observations on cows with severe hypokalemia (Group 2) justify the demand of the ethical animal committee of the Faculty for treatment of all members of the latter group.

6.4 Return of plasma potassium levels to normal in cows receiving an oral potassium supplementation and in untreated controls

Treatment options to replenish potassium in dairy cows include the intravenous administration of potassium in amounts that do not interfere with cardiac function. OETZEL (2007) observed that intravenous administration of potassium chloride solutions that were supposed to be safe failed to reconstitute physiologic plasma potassium levels in cows.

In animals suffering from DA the return to physiological plasma K^+ concentrations after surgery is caused on one hand by re-established transport of ingesta from the abomasum to the small intestine, the main site for K^+ absorption (KHORASANI et al., 1997). On the other hand, repositioning of the abomasum and omentopexy in the normal anatomical position results in a return of appetite and increase in feed intake shortly after surgery.

In this study, the administration of the bolus containing approximately 45-48 g potassium allowed reconstitution of mean plasma potassium levels to the reference range of 3.5-5.0 mmol/l in cows with moderate hypokalemia (plasma potassium level of ≥ 2.8 and < 3.5 mmol/l) suffering from periparturient diseases such as LDA, ketosis, metritis and mastitis within a short period of time (one hour following bolus administration). In untreated controls, the plasma potassium levels returned to the reference range of ≥ 3.5-5.0 mmol/l not earlier than following the return of the appetence after treatment of the underlying disease at approximately 36 hours after hospitalization.

The bolus formulation has proven its suitability for compensation of moderate hypokalemia in high yielding cows that show reduced appetite or even anorexia for various reasons. In this study, cows with severe hypokalemia received two or more boluses; however, the amount contained in the bolus was not always sufficient to replenish potassium stores, which may be due to the worse general condition in these animals, e.g. due to disturbances in glucose homoeostasis and acid-base balance as they suffered from lipomobilization syndrome. Oral administration of potassium chloride at a dosage of 100 g twice daily or even more to early lactating dairy cows (days 5 to 28 post partum) at risk (anorexia, ketosis) has been recommended by OETZEL (2007). CONSTABLE et al. (2014) examined the oral administration of potassium chloride to dairy cows following experimental induction of hypokalemia. Based on their findings the latter researchers judged oral treatment of cows with moderate hypokalemia with a dosage of 0.4 g KCl/kg body mass/24 hrs distributed over two treatments per day as sufficient and safe. SATTLER et al. (1998) proposed an amount of approximately 240 g per animal in 24 hours as treatment of severe hypokalemia. Due to its caustic effect, oral administration of potassium chloride in solution by use of a bottle or a drenching gun could cause irritation of the mucous membranes of the oral cavity and the esophagus. For this reason the administration of potassium chloride directly into the rumen by use of a stomach tube has been recommended. Under field conditions, however, the repeated use of a stomach tube is not a realistic option. Application of oral medications using a balling gun is clean, quick and easy. Balling guns exist as single-bolus instruments, as used in the present clinical trial, or as multiple-bolus guns. It is worthy of note, that these instruments may carry the risk of serious injury for the patient if used improperly. Therefore, knowledge of the cow's anatomy and fixation of the patient, as well as gentle introduction and lubrication of the instrument is necessary in order to prevent iatrogenic injuries of the oral cavity and larynx (DIVERS and PEEK, 2007). In the present study, application of the bolus formulation by the oral route using a balling gun proved to be an easy and safe procedure. On basis of the literature and our own observations, however, we recommend the administration of at least two boluses as initial treatment and repeated treatments at need.

6.5 Appetence/Feed Intake, Ruminal Movements and Milk Yield

Inappetence and hypokalemia as well as inappetence and decrease in milk yield are mutually dependent. Anorexia as a consequence of various production diseases causes hypokalemia. In the present study, all cows but one, which was seven months pregnant, were high yielding dairy cows in the transition period. Data that could be retrieved from the time before hospitalization demonstrated a history of decrease in milk yield. Unfortunately, no information regarding **feed intake** in the time before hospitalization and around calving could be ascertained. The maximum daily intake of concentrates over the course of the study was 4.5 kg per cow. Statistical analyses showed no significant differences in wet matter intake between the different groups but it was evident that cows with severe hypokalemia, that received at least two boluses, only gradually increased their daily feed intake over the observation period of 84 hours. The latter finding is in accordance with the low number of **ruminal contractions** auscultated in three minutes of animals in Group 2, as rumen motility patterns and the frequency of contractions depend on rumen fill and thus feed intake. Animals belonging to Group 2 had least ruminal contractions at 0 hrs (mean 1.2 contractions in 3 minutes) and a mean increase of 1.0 contraction per 3 minutes over the time span of 84 hrs. There were no significant differences in the increase in the number of ruminal contractions over time between the different groups, but descriptive statistics showed the least improvement of ruminal motility for control Group 1b, which supports the assumption of a role for potassium in rumen motility as has already been shown for abomasal muscle cells *in vitro* (ZURR and LEONHARD-MAREK, 2012).

Improvement in the clinical condition was associated with an increase in feed uptake and subsequent milk production, as there is a strong correlation between DMI and **milk yield** (BROWN et al., 1977; ST-PIERRE, 2008). No significant difference, however, was observed between the cows from the different treatment groups, although plasma potassium levels reached the lower limits of the reference range much earlier in Group 1a compared to Group 1b. For cows with severe hypokalemia (Group 2), the feed intake remained substantially lower than for cows with moderate hypokalemia at all times of observation, although the differences were not statistically significant, which may, however, have been attributable to the low number of animals in this group.

6.6 Position of the abomasum

The majority of cows included in the present study (16 (84.2 %) in Group 1a, 7 (63.6 %) in Group 2 and 14 (82.4 %) in Group 1b) were sent to the clinic because of DA. At admittance and during the morning examination on the following days, the position of the abomasum was evaluated by ultrasonographic examination from the right and left flank as well as the ventral abdominal wall (BRAUN et al., 1997; BRAUN, 2009). The underlying hypothesis for this examination was that the treatment with KCl might contribute to spontaneous remissions of abomasal displacement. *In vitro* studies demonstrated that KCl exerted a positive effect on abomasal contractility (TÜRCK and LEONHARD-MAREK, 2010; ZURR and LEONHARD-MAREK, 2012). For this reason administration of KCl was thought to increase abomasal motility and by this mechanism could contribute to a faster transport of abomasal contents and gas into the intestines.

Results of this study were not sufficient to support the aforementioned hypothesis, as in patients suffering from LDA, individual varieties in abomasal position, which may have been arising from anatomical differences in the patients as well as a varying degree of gas accumulation in the organ, confounded a diagnostically conclusive comparison of individuals in the relatively low number of study animals, keeping in mind that the technique described

by BRAUN et al. (1997) was used to investigate the abomasum in its physiological position. Furthermore, as mentioned before, all cows suffering from LDA underwent surgical correction the day after hospitalization in accordance with the standard operating procedures of the clinic. The influx of air into the abdominal cavity during laparotomy and the resulting pneumoperitoneum hindered ultrasonographic examination in the following days.

Complete daily measurements of eight cows without LDA showed no significant differences in abomasal position or extension between the groups over the study period. Measurements of the cranial and caudal lateral extension were greater on the right than on the left side, which is in accordance with findings of BRAUN et al. (1997) and WITTECK et al. (2005). Changes in length, lateral extension and position were most likely attributable to the physiological motility of the abomasum and could not statistically be associated with the administration of the bolus. Noteworthy, the cows included in the present study were second line patients referred by local practitioners to the clinic after conservative treatment had not been successful. To this end, it could be worth to examine the effect of administration of potassium boluses to freshly diagnosed cows suffering from LDA in the field and evaluate if spontaneous remissions occur in these cows.

6.7 Energy metabolism

Due to the sudden onset of lactation and the rumen volume being not large enough to compensate for energy demands for maintenance and milk production by increases of dry matter intake, the dairy cow experiences a period of negative energy balance. The cow has to invest part of her body reserves such as muscle glycogen, glucogenic amino acids as well as her body fat reserves to compensate for the negative energy balance (HERDT, 2000). Risk factors such as obesity or cachexia and disorders that cause anorexia have been shown even to increase the existing energy deficit. Various alterations have been reported for cows in the transition period. The mobilization of body fat is reflected in increased plasma levels of non-esterified fatty acids (NEFA > 0.7 mmol/l for cows in the transition period), which are normally oxidized in the ß-oxidation or exported by the liver after having been incorporated in transport proteins. When too much **NEFA** reach the liver, part of these are re-esterified to triacylglycerol (TAG) and stored inside the liver cell (GRUMMER, 1993). Accumulation of more than 30 % liver TAG on wet weight basis has in extreme cases been associated with hepatic encephalopathy as well as liver failure (BOBE et al., 2004). When gluconeogenesis is impaired in anorectic cows, the blood **glucose** levels decrease, which triggers the production of ketone bodies, an efficient glucose sparing mechanism in ruminants. Whenever blood levels of **ß-hydroxybutyrate** exceed 1.2 mmol/l (subclinical ketosis), however, dairy cows are at increased risk for developing additional disorders (RABOISSON et al., 2014), and blood levels above 3.0 mmol/l are indicative for clinical ketosis. In the present study, increased glucose levels were observed at the day of hospitalization, which presumably were a consequence of the stress response elicited by the fact that the animals had been separated from their group and transported to the clinic. The day after hospitalization glucose levels decreased to the normal range again. Mean NEFA and ß-hydroxybutyrate levels, however, were substantially increased in animals from all groups.

NEFA and ß-hydroxybutyrate levels and blood gas analysis in animals from Group 2 revealed substantial lipomobilization and severe ketoacidosis. In humans with ketoacidosis due to type 2 diabetes, which resembles the situation of an over-conditioned cow entering the transition period and developing a secondary ketosis, potassium depletion via the kidney was observed as a consequence of osmotic diuresis (CHIASSON et al., 2003). Infusions consisting of insulin, glucose and potassium were shown to reduce NEFA levels by inhibition

of lipolysis from body fat depots and to improve the energy metabolism of myocardial cells following open heart surgery in man (SZABO et al., 2001). The effect was not observed following insulin injections alone. Following administration of potassium, CONSTABLE et al. (2014) demonstrated a reduction in NEFA levels in transit cows with experimentally induced hypokalemia. Although – as in the paper from CONSTABLE et al. (2014) – in the present study statistical power was not sufficient to deliver significant differences between cows treated with potassium and controls, when looking at individual results of cows treated with one bolus of potassium, we also observed a steeper decrease in plasma NEFA levels compared to controls, indicating that fat mobilization may be reduced in cows treated with KCl. Observations in humans indicate that potassium in the diet could reduce the risk of diabetes (CHATTERJEE et al., 2011). In addition, potassium seems to have an effect on membrane channels that are involved in insulin secretion. Insulin secretion has been shown to decrease ketogenesis by inhibition of lipolysis. To this end, it could be of interest to examine the insulin response following potassium administration in transit cows.

6.8 Acid-base Balance

In terms of acid-base balance the groups were very heterogeneous, which can lead to misinterpretation of the results. Animals with LDA are known to tend to alkalosis, although recent observations show, that there are many cows suffering from severe acidosis due to prolonged anorexia, liver failure and ketoacidosis. Normally, H^+ and Cl^- accumulate in the abomasum, while Na^+ and HCO_3^- remain in the extracellular space. As long as Cl^- is reabsorbed in the lower small intestine in exchange for HCO_3^- normal blood pH is maintained (GRÜNDER, 2006). Abomasal outflow disorders and obstruction of the proximal duodenum result in abomasal reflux syndrome characterized by hypochloremic metabolic alkalosis often accompanied by hypokalemia (BREUKINK and KUIPER, 1980).

When acidemia occurs, intracellular buffering of H^+ ions in exchange for K^+ as well as hampering of the Na^+/K^+-ATPase has been thought to lead to a rise in extracellular K^+ concentration, and, especially if renal function does not suffice to eliminate excess K^+, result in hyperkalemia. In the present study however, cows belonging to Group 2 presented with severe hypokalemia ($K^+ < 2.8$ mmol/l) despite being acidotic. Interestingly, the steeper decline in pH of Group 2 when compared to the other groups was especially distinct at 12 and 36 hours following administration of K^+, when mean plasma potassium levels had already been restored to a level above 3.3 mmol/l, and the administration of K^+ to cows of Group 2 did not result in such a steep increase in the blood levels of K^+ as observed in cows belonging to Group 1a. In all but one study animal showing a negative base excess over the whole study period, potassium levels failed to be reconstituted to normal levels. In addition, the improvement of the clinical condition (feed intake, ruminal motility) was more evident in animals with moderate hypokalemia (Group 1a).

Various authors describe the implications of metabolic acidosis and diabetic ketoacidosis for the reconstitution of K^+ in hypokalemic animals and man (ADLER and FRALEY, 1977; FUBINI and DIVERS, 2007; ARONSON and GIEBISCH, 2011; CIOBOTARU, 2013; DAVIS et al., 2016). Osmotic diuresis is observed in humans with ketoacidosis leading to reduced blood pressure and activation of the RAAS. To this end, Na^+ is reabsorbed in the kidney in exchange for H^+ and K^+ ions which aggravates the hypokalemia. In addition, the presence of H^+ forces these cations into the cell and Na^+ out of the cells (H^+/Na^+ exchanger) causing intracellular acidosis. Intracellular acidosis has been shown to hamper the function of the Na^+/K^+-ATPase, such that K^+ cannot enter the intracellular space (ADLER and FRALEY, 1977; ARONSON and GIEBISCH, 2011).

The dosage of K^+, although two boluses were administered, was not sufficient to reconstitute the plasma K^+ concentration in all cows included in Group 2. Intracellular K^+ depletion, perhaps due to prolonged periods of decreased dietary intake or following the efflux of K^+ due to a current (keto-) acidotic state by mechanisms as described earlier, would provide an explanation for this observation. In addition, the Cl^- anion from the bolus could also have contributed to intracellular acidosis, promoting K^+ efflux and intracellular K^+ depletion due to malfunction of the Na^+/K^+-ATPase. However, this could not be proven in the present study by analysis of muscle K^+ content. Our observations in severe hypokalemic and acidotic cows nevertheless plead for administration of greater amounts of K_2CO_3 instead of KCl in order to reconstitute the intracellular acid-base equilibrium such that the Na^+/K^+-ATPase is able to fulfill its function in transporting the administered K^+ to the intracellular space. Our findings are in accordance with other authors who also recommend the administration of K_2CO_3 to dairy cows in the transition period instead of potassium chloride due to the unwanted acidifying effect of chloride (HARRISON et al., 2010).

The administration of K^+ had no significant effect on plasma Na^+ concentrations neither with time, nor between different treatment groups. Increased plasma levels of Ca^{2+} were most prominent in cows from Group 2 over the whole observational period of 84 hours, which might possibly be due to metabolic acidosis as demonstrated by blood gas analysis.

6.9 Potassium in muscle biopsy samples

Plasma potassium levels do not necessarily reflect total body stores of the element. This is especially true for conditions that affect the acid-base homeostasis as reflected by the strong ion theory. JOHNSON et al. (1991) described, that in healthy, adult horses, whole body potassium depletion did not significantly change plasma potassium concentration. Erythrocyte potassium concentration however decreased in the first 48 hours and thereafter did not change and potassium concentration of the middle gluteal muscle still decreased on day 7 of the study. In the latter study no correlation was observed between plasma and erythrocyte K^+ concentration or plasma and middle gluteal muscle K^+ concentration. The results indicate that plasma K^+ concentration cannot be used as an index for the whole body K^+ content, whereas the intracellular potassium concentration in muscle cells seems to represent whole body K^+ status (JOHNSON et al., 1991).

These findings are consistent with several studies, which show that intra-erythrocyte potassium concentrations are not suitable for estimation of intracellular K^+ concentrations elsewhere in the body (CHRISTINAZ and SCHATZMANN, 1972; CARLMARK et al., 1982). SCHNEIDER et al. (2016) even showed significant differences ($p < 0.001$) in K^+ levels in muscle tissue and erythrocytes. Consequently, the best indicator for the K^+ pool of the body is the K^+ content of skeletal muscle tissue.

In the work of EL-ZAHAR (2015) and in the present study, a procedure to ascertain information on whole body K^+ status in cows and its alteration after treatment of hypokalemic cows with KCl by muscle biopsy was optimized. In first instance, biopsies using a modified *Bergström* needle were obtained by the technique described by BERGSTRÖM and HULTMAN (1997). This technique, however, delivered biopsies that were unsuitable for analysis due to artifacts caused by the sampling procedure as demonstrated by histological examination of biopsy material. To this end, we developed a technique for obtaining muscle biopsies from cattle by a modified *Bergström* technique using vacuum on the modified *Bergström* needle in order to draw the tissue into the lumen of the needle (HENNESSEY et al., 1997; LEITNER, 2009). The suction technique resulted in intact biopsy material and the

biopsy material was shown to be pure muscle tissue without substantial amounts of connective tissue or fat as verified by histological examination

Skeletal muscle biopsy samples were obtained from nine of the animals enrolled in the present study and in the same time frame from 13 third party animals in the study performed by EL-ZAHAR (2015) (patients that had to be euthanized for various reasons due to unfavorable prognosis, but did not suffer from disorders affecting the metabolism).

In both studies, the results from analysis by AAS delivered great variations in potassium contents in muscle tissue between animals. The levels, however, were in accordance with the range reported for cattle by other researchers (CONSTABLE et al., 2014). Biopsies of euthanized animals demonstrated no correlation between blood K^+ and muscle K^+ contents ($r = 0.06$), and between blood pH and muscle K^+ contents ($r = 0.01$). The administration of KCl did not deliver conclusive results with respect to intramuscular K^+ contents. This finding might be a consequence of the low number of animals sampled and differences in acid-base status in these animals ranging from normal pH to severe metabolic acidosis. With the suction assisted biopsy technique, a sampling method becomes available that allows sampling in a greater number of animals in experimental settings in order to elucidate potassium metabolism in healthy and diseased cattle.

6.10 Patient history and outcome

Data that were retrieved for cows which returned home showed that most of the animals got back into lactation after their return and 86.5 % of cows for which follow-up data was provided were still on their farms of origin one month after completion of the study. Only 4 animals were not on their farm of origin anymore due to exitus letalis or euthanasia. 6 animals were slaughtered or sold and the remaining 27 animals were still in service. Our observations are in accordance with other researchers who observed a fair prognosis for dairy cows following surgical treatment of DA. STJEAN (1990) reported that following surgery for LDA, 9 – 17 % of the cows did not finish the lactation. In the present study, 33.3 % of the cows in Group 1a, 21.4 % of cows in Group 1b and 25 % of cows in Group 2 did not finish the lactation subsequent to hospitalization and 13.3 % of Group 1a cows, 7.1 % of Group 1b animals and 25 % of Group 2 animals left the farm within less than 28 days after return to their farms of origin. Although not all of the study animals underwent abdominal surgery, the percentages of animals not getting back into lactation were higher in our present study than described by STJEAN (1990). The study animals, however, represent a preselected population of cows that had been referred to the animal hospital by local practitioners. As reflected by increased blood levels of NEFA and ß-hydroxybutyrate at admission, most of the animals in the present study were mobilizing substantial amounts of body fat from their stores in order to compensate for negative energy balance. The assumption of potassium having a great impact on energy metabolism in humans (LEE et al. 2013) has been reported also for milk production in dairy cows (JENKINS et al., 2010). The exact mechanism, however, has not been elucidated yet but might include insulin secretion. Our observations did not suffice to verify the assumption of CONSTABLE et al. (2014) that potassium might be able to regulate lipolysis in transition cows. Earlier studies in dairy cows with LDA hospitalized in the Clinic for Ruminants and Swine demonstrated that the higher the liver fat content in patients with DA was, the lower was the prognosis. Triacylglycerol contents in the liver above 33 % were related to poor prognosis (AHMED, 2004).

According to patient history data ascertained from the herd managers after completion of the study, only three cows assorted to Group 1a and 1b, respectively, and no cow assorted to Group 2 had a milk yield of more than 30 kg/day at the point of entry in the study. Although in

the present study no statistically significant positive effect of oral potassium substitution on milk yield of cows with hypokalemia was observed, the initially slightly steeper increase in milk yield as observed for the animals of the treatment group (1a) in contrast to controls could be a reflection of a positive effect of potassium supplementation on milk yield, which is in line with observations by HARRISON et al. (2011). The improvement in the general condition of the probands, however, going alongside with the increase in feed intake very likely contributed to a greater extent to the increase in milk production. For a population comparable to the animals in the present study BAR and EZRA (2005) calculated total milk losses for ketosis being 96.1 kg for first lactation cows, 151.5 kg for second lactation cows and 234.8 kg for third lactation cows, respectively. MCART et al. (2011) observed higher milk yields between 1.34 kg/d and 1.59 kg, respectively, in cows with subclinical ketosis treated with propylene glycol compared to untreated controls on two different farms. For cases of LDA a loss in milk production of 250 to 500 kg in the year of surgery was reported by STJEAN (1990). The results of the follow-up questionnaire of the present study might reflect the faster recovery following oral substitution of potassium in hypokalemic cows as nine cows from in total 15 belonging to the treatment groups 1a and 2 had a milk yield of more than 30 kg/day after return on their farms of origin (60%) whereas the same was true for only three cows from 10 belonging to the control Group 1b (30%).

7 CONCLUSIONS

Results of this study showed, that a bolus formulation containing potassium may present an effective and feasible alternative to the application of dissolved KCl administered per nasogastric tube by the dairy farmer or local practitioner. Furthermore, a new method for muscle biopsy sampling was developed and successfully implemented.

In cows with moderate hypokalemia (K^+ ≥ 2.8 mmol/l and < 3.5 mmol/l) the return of potassium levels to the reference range (K^+ ≥ 3.5 mmol/l) was faster in those receiving the bolus, than in untreated controls. Despite repeated administration of up to three boluses on subsequent days as well as rescue treatment in single cases, animals with severe hypokalemia (K^+ < 2.8 mmol/l) had a less favorable prognosis when compared to cows with moderate hypokalemia and were in need of a longer period of convalescence. Differences in an increase in milk yield could be observed between treated animals and untreated controls and were apparent in follow-up data received from the herd owners after completion of the study; however, the results regarding milk yield, as well as vaginal discharge characteristics and the position of the abomasum as determined by ultrasound, could not sufficiently be secured statistically due to the low number of probands. To this end, the effects of the potassium bolus in the recovery period of disease should be studied in a greater number of anorectic cows versus untreated controls. Since potassium plays a major role in all kinds of physiological processes in the body, the restitution of normal potassium concentration has to be considered being of great importance for the convalescence of hypokalemic individuals, especially those suffering from diseases that are known to influence energy and electrolyte metabolism. The daily analysis of blood parameters is not possible for local practitioners and cow-side analysis of potassium concentration is yet to be developed. Therefore it is of great importance, that possibly hypokalemic animals suffering from diseases that have been shown to be associated with hypokalemia can be treated quickly and safely without risk of overdosing.

Even repeated administration of the potassium bolus formulation applied in the present study had no negative side effects on study animals. In view of the lack of adverse reactions and as determination of potassium in a timely manner is not always possible on farm, cows in the transition period, especially those showing decreased feed intake, signs of muscle weakness, muscle fasciculation, lassitude or fatigue in absence of an alternative explanation, may profit from receiving one bolus prophylactically. The results for cows with severe hypokalemia, however, demonstrated that the latter animals need higher dosages of potassium and for this reason should be treated initially with two boluses of the kind we administered, following determination of plasma potassium values. Furthermore, especially in hypokalemic cows with (keto-)acidosis, the efficacy of a formulation containing potassium carbonate should be investigated due to its alkalinizing effect.

8 SUMMARY

Effects of Oral Supplementation of Potassium Chloride in Hypokalemic Dairy Cows by Use of a Bolus Formulation on Metabolism, Abomasal Position and Vaginal Discharge Characteristics

Dairy cows that revealed moderate hypokalemia (plasma potassium levels between ≥ 2.8 and < 3.5 mmol/l) when admitted to the animal hospital were by chance assigned either to a treatment group (Group 1a: 19 cows) or to a control group (Group 1b: 17 cows), while all animals with severe hypokalemia (plasma potassium levels < 2.8 mmol/l; Group 2: 11 cows) were treated with potassium for ethical reasons, as they showed severe derailment of potassium homeostasis indicative for a severely impaired general condition. The study animals had been referred to the Ruminant and Swine Clinic, Department of Veterinary Medicine, Freie Universität Berlin for various diseases including abomasal displacement to the left (LDA), ketosis, metritis and mastitis occurring as single entities or in combination.

Cows enrolled in the treatment groups (1a and 2) were orally administered potassium in a bolus formulation by use of a balling gun while animals allotted to Group 1b remained untreated and served as controls. The bolus formulation contained approximately 85-91 g potassium chloride and 15-16 g magnesium oxide. Blood samples were obtained for blood gas analysis and the determination of plasma levels of potassium, sodium, glucose, ionized calcium before and at 1, 2, 3, 12, 36, 60 and 84 hours following bolus or sham administration (Group 1b controls), respectively. ß-hydroxybutyrate, aspartate transaminase (AST), alkaline phosphatase (AP), non-esterified fatty acids (NEFA), albumin, bilirubin, total calcium, total magnesium, creatinine and blood urea nitrogen (BUN) were determined at admission and at dismissal (84 hours). A clinical examination following a standard examination protocol was performed each morning by the same person during the observational period of five calendar days and at dismissal. Ultrasonography was performed in regular intervals of up to 12 hours following admittance. In animals that were referred to the clinic for suspected LDA, ultrasonographic assessment was performed in order to find out whether treatment with potassium would lead to enhanced motility and emptying of gas from the abomasum and to this end would contribute to relocation of the organ into its normal anatomical position, however, pneumoperitoneum following surgical correction of LDA, or abdominal fat in some cases, hindered further evaluation of ultrasonographic data in the majority of cows. Daily milk yield and feed intake were recorded. We developed a technique for suction assisted muscle biopsy by modifying the original procedure described by BERGSTRÖM and HULTMAN (1997). Diagnostic muscle biopsies were obtained to evaluate total body potassium status from nine animals and third party controls.

Oral administration of potassium using a bolus formulation proved to be safe and easy. In cows with moderate hypokalemia (Group 1a) bolus administration resulted in reconstitution of mean and medium plasma potassium levels to normal (reference range 3.5-5.0 mmol/l) within one hour following administration, whereas the same was true for untreated controls (Group 1b) at 36 hours after admission. In the group of severely hypokalemic cows (Group 2), where all animals received a treatment for ethical reasons, the mean potassium level passed the lower border of the reference range after 36 hours.

Bolus administration in cows with moderate hypokalemia (Group 1a) did not result in significant differences compared to sham treated controls (Group 1b) with respect to plasma levels of NEFA, ß-hydroxybutyrate, glucose, sodium, blood urea nitrogen, creatinine, albumin, bilirubin, calcium, magnesium, AST and AP at day five following administration of

the bolus. The variables rather reflected the stabilization of the metabolism within the recovery period for animals from all groups.

Due to the importance of potassium for body functions and vital processes animals at risk such as anorectic high-yielding dairy cows should be treated with potassium as soon as possible in order to prevent unwanted side effects of hypokalemia such as muscle weakness or even recumbency as described in scientific literature. Due to its alkalinizing effect, which could especially be of use in severely hypokalemic cows with acidosis, the development of a bolus containing potassium carbonate should be considered.

9 ZUSAMMENFASSUNG

Auswirkungen der oralen Verabreichung von Kaliumchlorid an hypokaliämische Milchkühe mittels eines Bolus auf Stoffwechsel, Position des Labmagens und Beschaffenheit des Vaginalsekretes

Milchkühe mit geringgradiger Hypokaliämie (Plasmakaliumkonzentration unter 3,5 mmol/l) bei Einweisung in die Tierklinik wurden randomisiert einer Behandlungsgruppe (Gruppe 1a: 19 Kühe) bzw. einer Vergleichsgruppe (Gruppe 1b: 17 Kühe) zugewiesen, wohingegen alle Tiere mit schwerwiegender Hypokaliämie (Plasmakaliumkonzentration < 2,8 mmol/l; Gruppe 2: 11 Kühe) aus ethischen Gründen mit Kalium substituiert wurden. Die Tiere waren zuvor wegen verschiedener Erkrankungen wie linksseitiger Labmagenverlagerung, Ketose, Metritis oder Mastitis, alleine oder in Kombination, an die Klinik für Klauentiere des Fachbereichs Veterinärmedizin der Freien Universität Berlin überwiesen worden.

Kühe der Behandlungsgruppen (1a und 2) erhielten Kalium peroral mittels eines Bolus verabreicht. Der Bolus enthielt etwa 85-91 g Kaliumchlorid sowie ca. 15-16 g Magnesiumoxid. Bei Kühen der Vergleichsgruppe 1b wurde die Bolusgabe lediglich mit Hilfe des Eingebers simuliert. Blutproben zur Analyse von Blutgasen und den Plasmakonzentrationen von Kalium, Natrium, Glukose und ionisiertem Kalzium wurden vor der Bolusgabe sowie 1, 2, 3, 12, 36, 60 und 84 Stunden nach Verabreichung bzw. simulierter Verabreichung gewonnen. Die Werte von BHBS, AST, AP, NEFA, Albumin, Bilirubin, Totalkalzium, Totalmagnesium, Kreatinin und BUN wurden bei Einweisung in die Klinik sowie am Ende des Untersuchungszeitraumes (84 h) untersucht. Die klinische Untersuchung wurde anhand eines standardisierten Untersuchungsprotokolls jeden Morgen des Untersuchungszeitraumes von 5 Kalendertagen sowie vor Entlassung aus der Studie durch dieselbe Person durchgeführt. Die Lage des Labmagens wurde mittels Ultraschall in regelmäßigen Abständen während der ersten 12 Stunden nach Einstallung kontrolliert. Bei Tieren, die wegen des Verdachts auf linksseitige Labmagenverlagerung eingestallt worden waren, wurde die Ultraschalluntersuchung durchgeführt um zu überprüfen, ob die Verabreichung von Kalium zu einer vermehrten Kontraktilität des Labmagens und einer Gasentleerung und somit zur spontanen Verlagerung des Organs in seine physiologische Position beitragen könnte. Es zeigte sich jedoch, dass durch das postoperative Auftreten eines Pneumoperitoneums bei operierten Tieren sowie eine bei einigen Kühen vorhandene erhebliche Fettschicht die weitere Analyse der Ultraschalldaten bei einem Großteil der Tiere verhinderte. Die tägliche Milchmenge und Futteraufnahme wurde dokumentiert. Eine Modifikation des von BERGSTRÖM und HULTMAN (1997) beschriebenen Verfahrens zur Entnahme von Muskelbioptaten wurde entwickelt, bei welchem wir Probenmaterial mit Hilfe eines manuell erzeugten Vakuums gewinnen konnten. Muskelbioptate wurden von 9 Tieren entnommen, um das Gesamtkalium zu bestimmen.

Die Verabreichung von Kalium mittels Bolus war sicher und einfach. Bei Tieren mit moderater Hypokaliämie (Gruppe 1a) normalisierte sich die durchschnittliche und mediane Plasmakaliumkonzentration innerhalb einer Stunde nach Verabreichung des Bolus (Referenzbereich: 3.5-5.0 mmol/l). Bei der Vergleichsgruppe (Gruppe 1b) wurde der Referenzbereich nach 36 Stunden erreicht. Bei Gruppe 2, den Tieren mit hochgradiger Hypokaliämie, in welcher alle Tiere Kaliumboli erhalten hatten, erreichte die durchschnittliche Kaliumkonzentration den Referenzbereich ebenfalls nach 36 Stunden.

Die Plasmakonzentrationen von NEFA, BHBA, Glukose, Natrium, BUN, Kreatinin, Albumin, Bilirubin, Kalzium, Magnesium, AST und AP unterschieden sich 5 Tage nach Verabreichung des Bolus nicht signifikant zwischen den Gruppen 1a (leichte Hypokaliämie) und 1b (Kontroll-

gruppe). In allen Gruppen zeigte sich hingegen eine Stabilisierung des Stoffwechsels innerhalb der Zeitspanne des Versuchs.

Aufgrund der Bedeutung von Kalium für viele Körperfunktionen und lebenswichtige Prozesse sollte Tieren, die Gefahr laufen, eine Hypokaliämie zu entwickeln, wie beispielsweise anorektischen hochleistenden Milchkühen, so früh wie möglich Kalium verabreicht werden, um den aus der Literatur bekannten unerwünschten Effekten der Hypokaliämie, wie Muskelschwäche und Festliegen, vorzubeugen. Wegen seiner alkalisierenden Wirkung, welche besonders bei hochgradig hypokalämischen Kühen mit Azidose von Nutzen sein könnte, sollte die Entwicklung eines Kaliumkarbonat enthaltenden Bolus in Betracht gezogen werden.

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11 APPENDIX

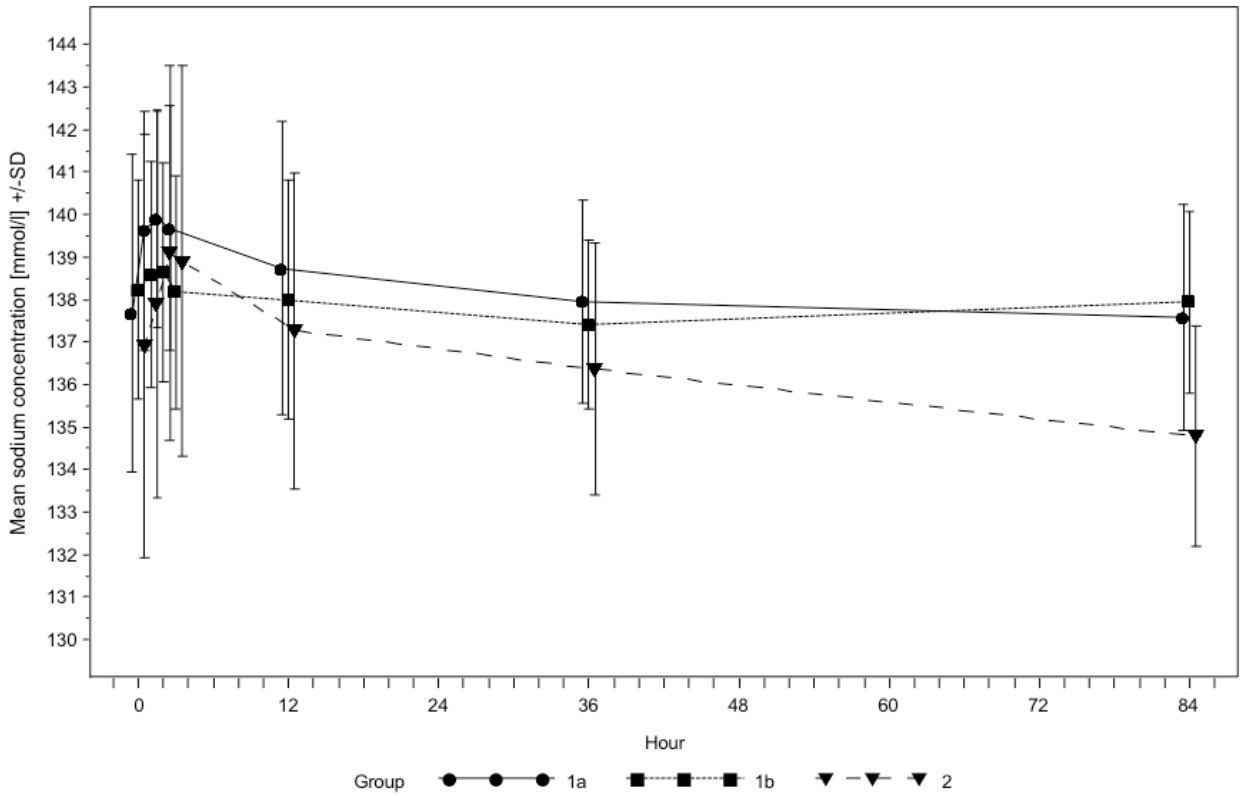

Fig. 36. Time course of mean plasma sodium concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at different time points following bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

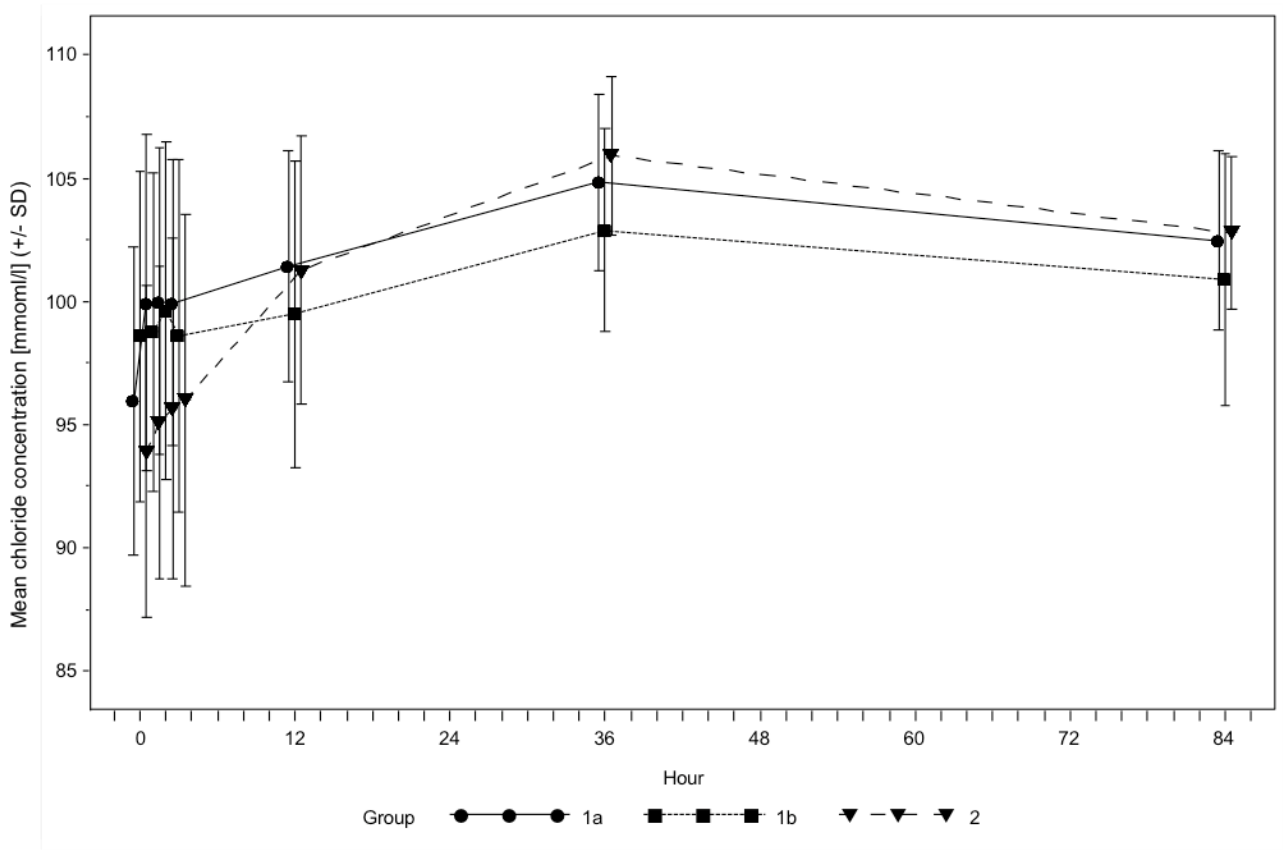

Fig. 37. Time course of mean plasma chloride concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at different time points following bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

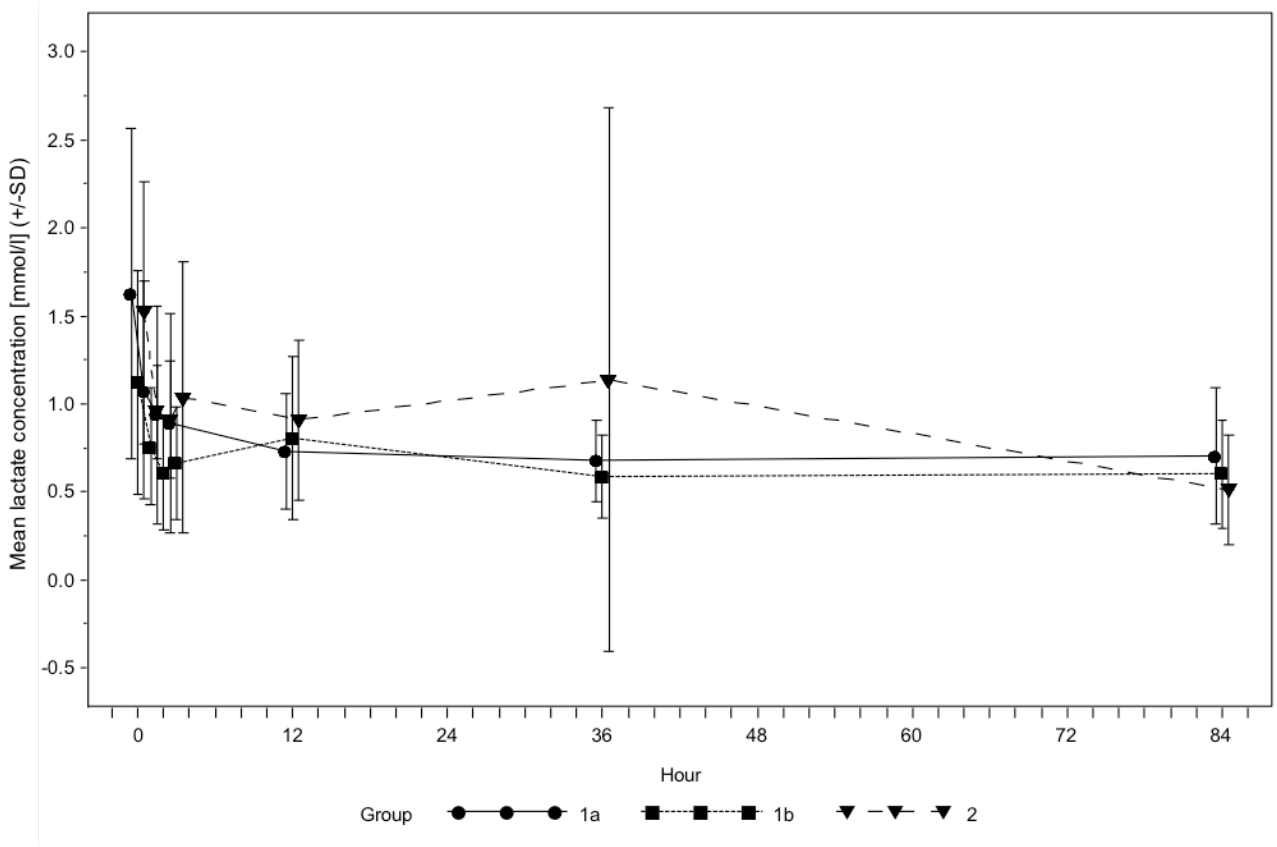

Fig. 38. Time course of mean blood lactate concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at different time points following bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

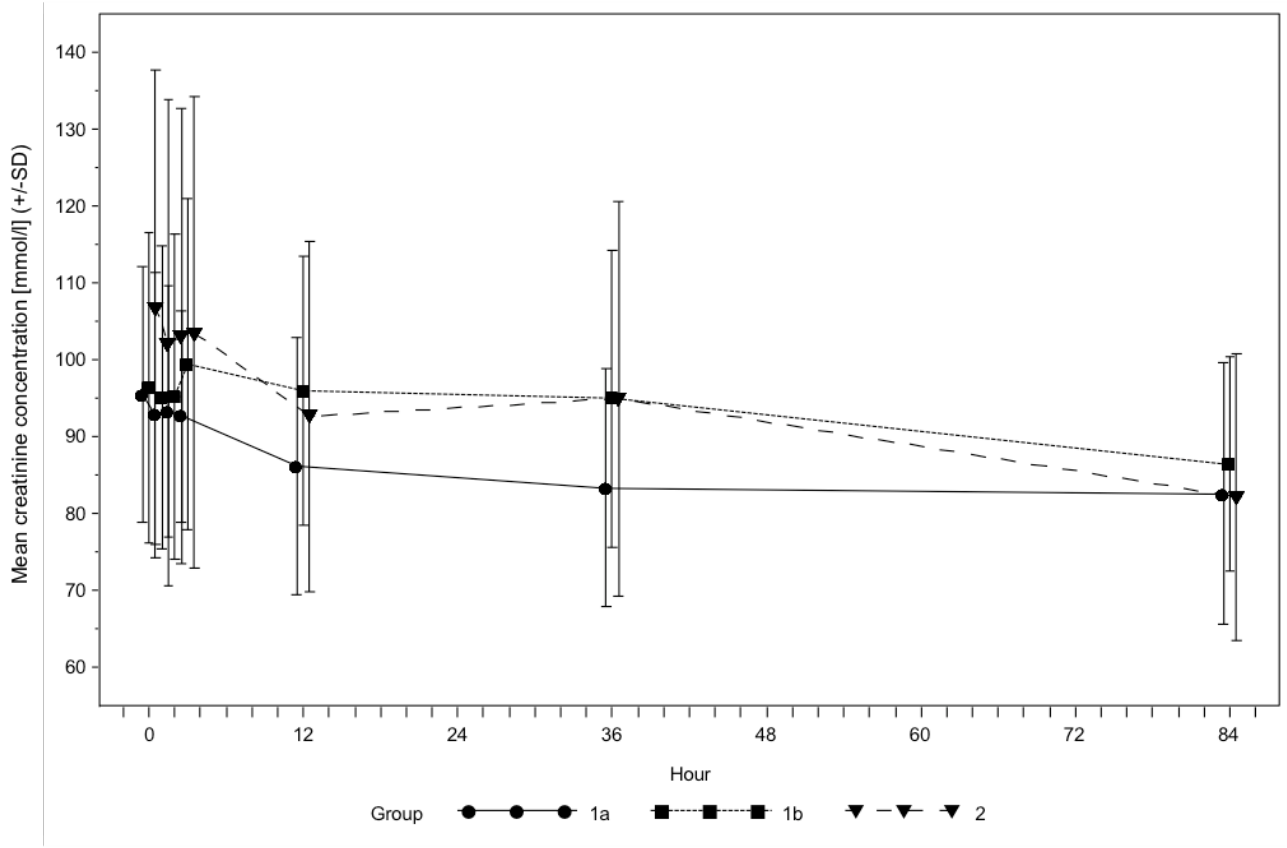

Fig. 39. Time course of mean blood creatinine concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and at different time points following bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

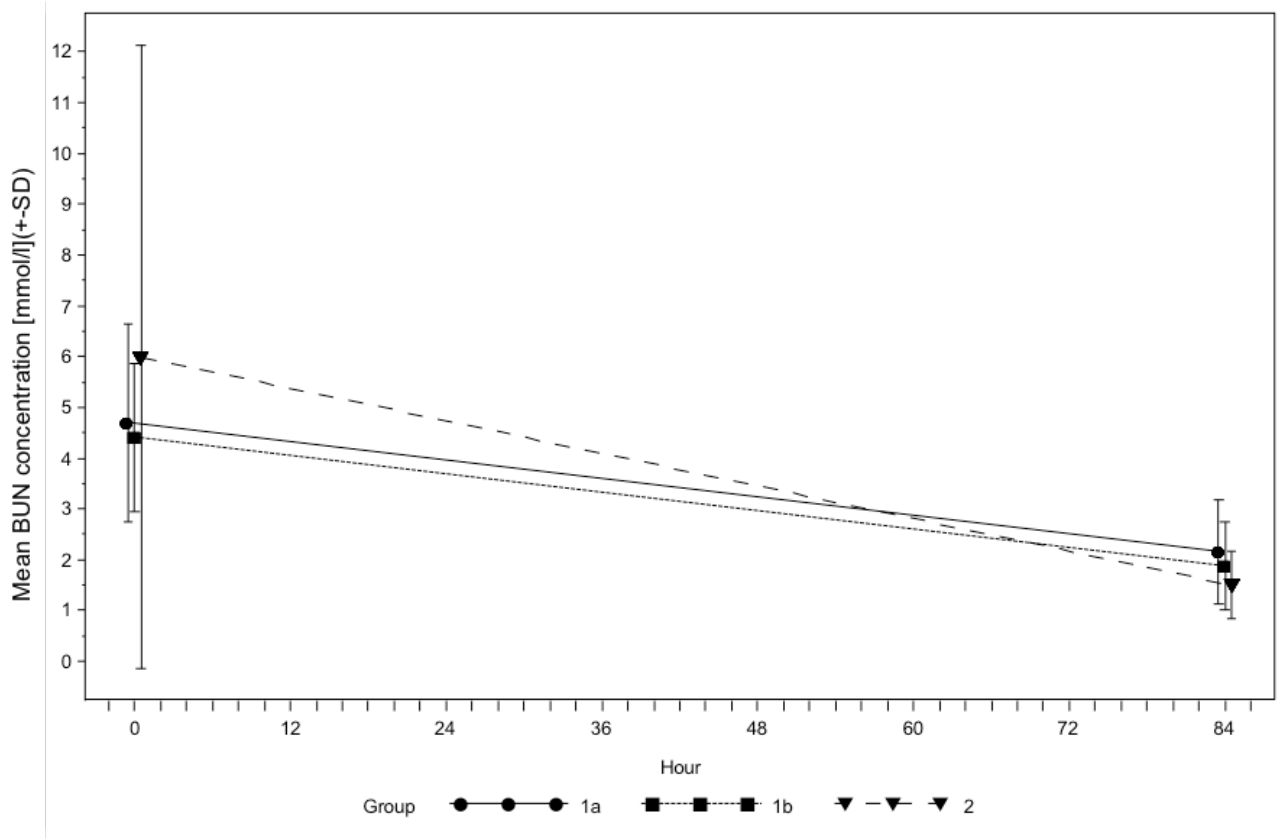

Fig. 40. Time course of mean blood urea nitrogen (BUN) concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

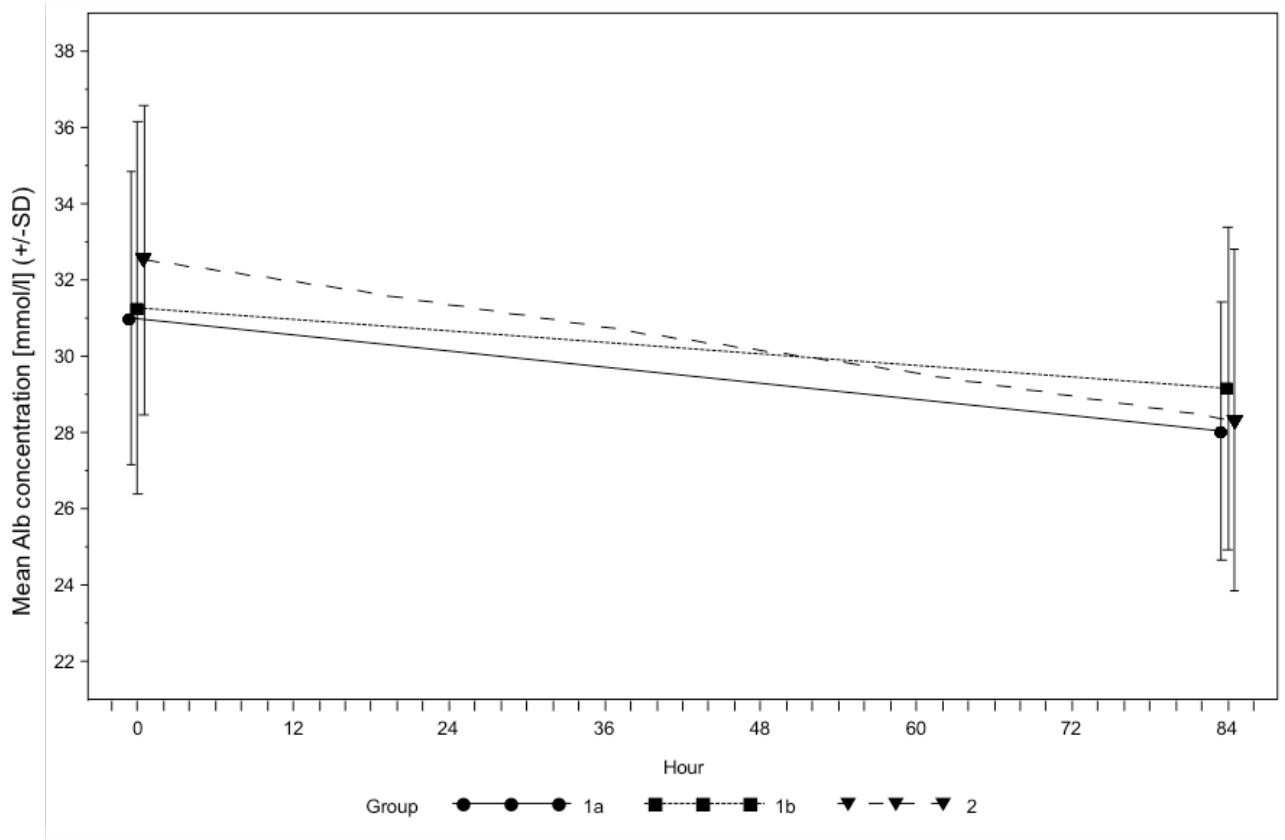

Fig. 41. Time course of mean albumin (Alb) concentrations [g/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

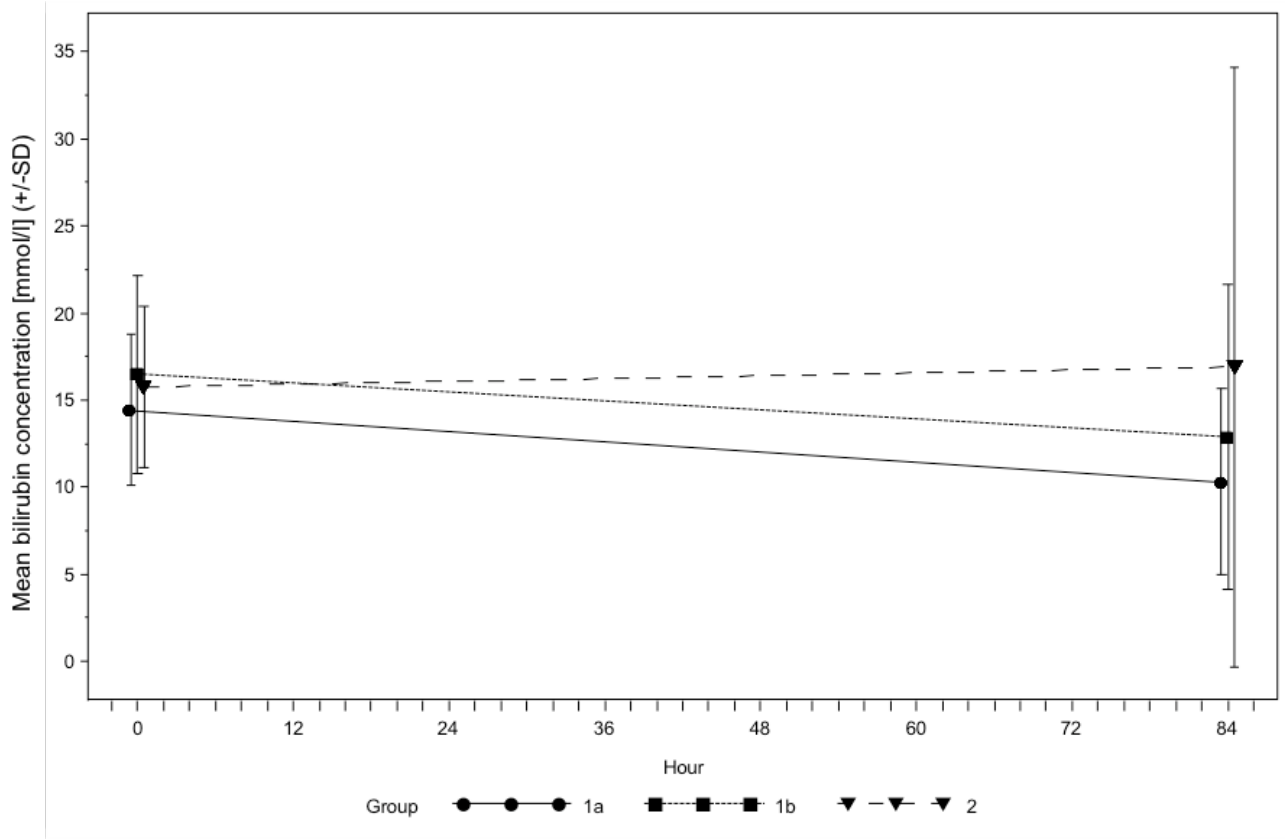

Fig. 42. Time course of mean bilirubin concentrations [µmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

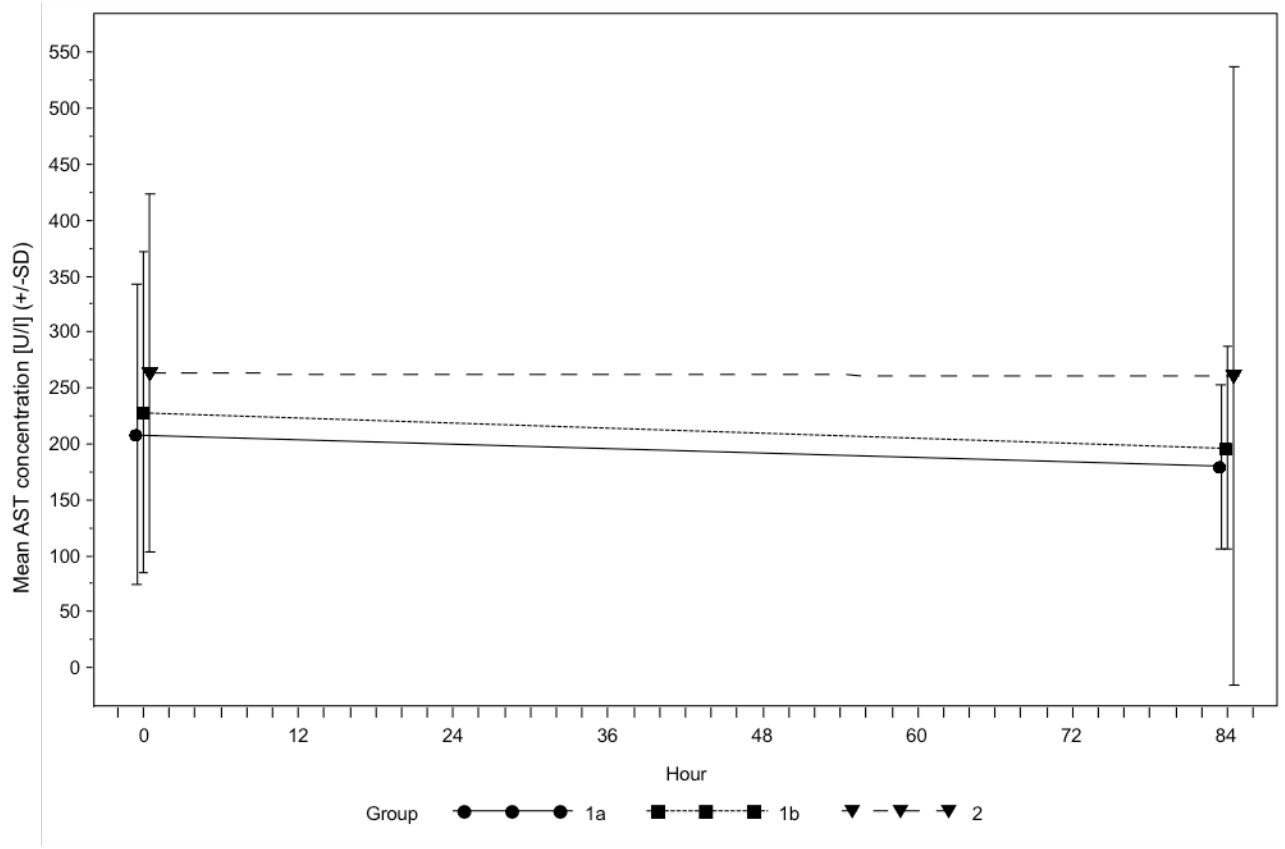

Fig. 43. Time course of mean aspartate aminotransferase (AST) level [U/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

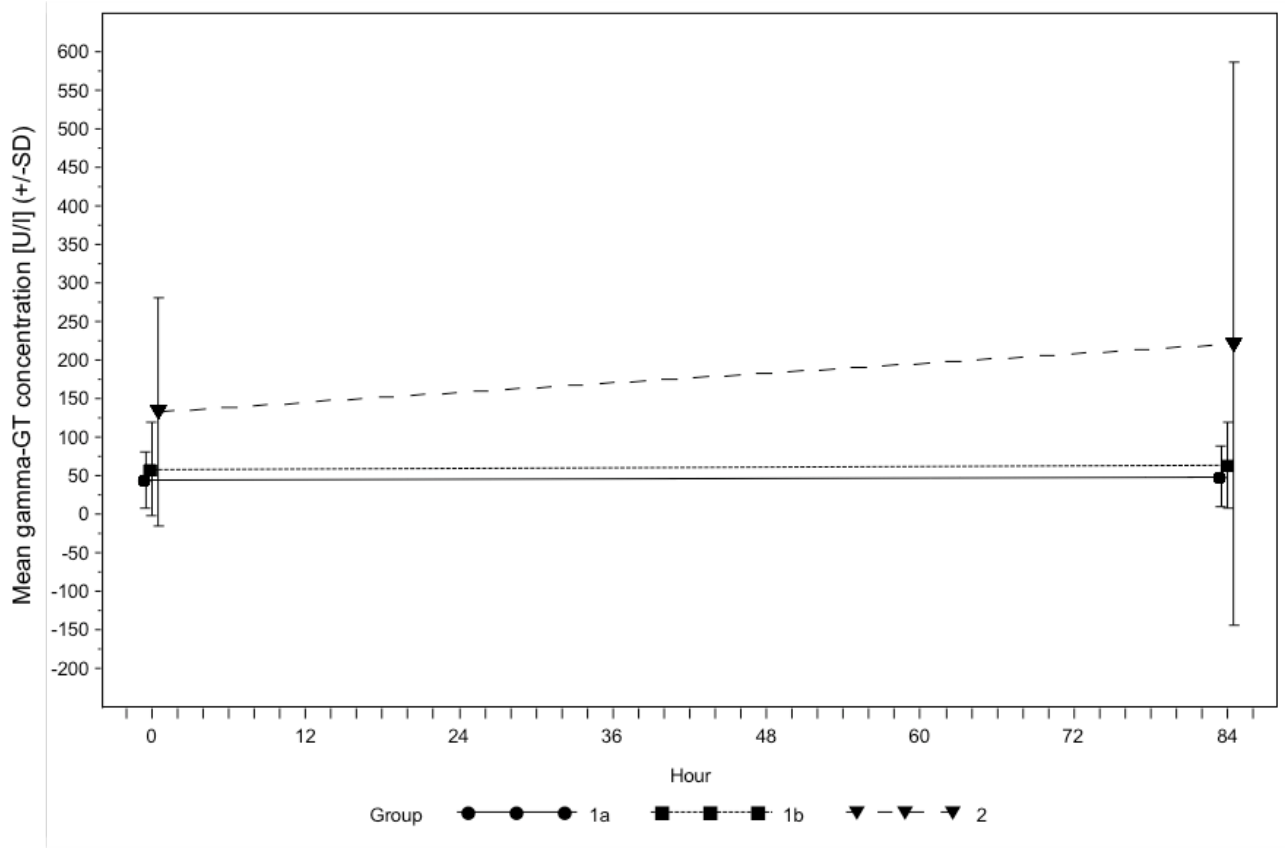

Fig. 44. Time course of mean gamma-glutamyltransferase (gamma-GT) level [U/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

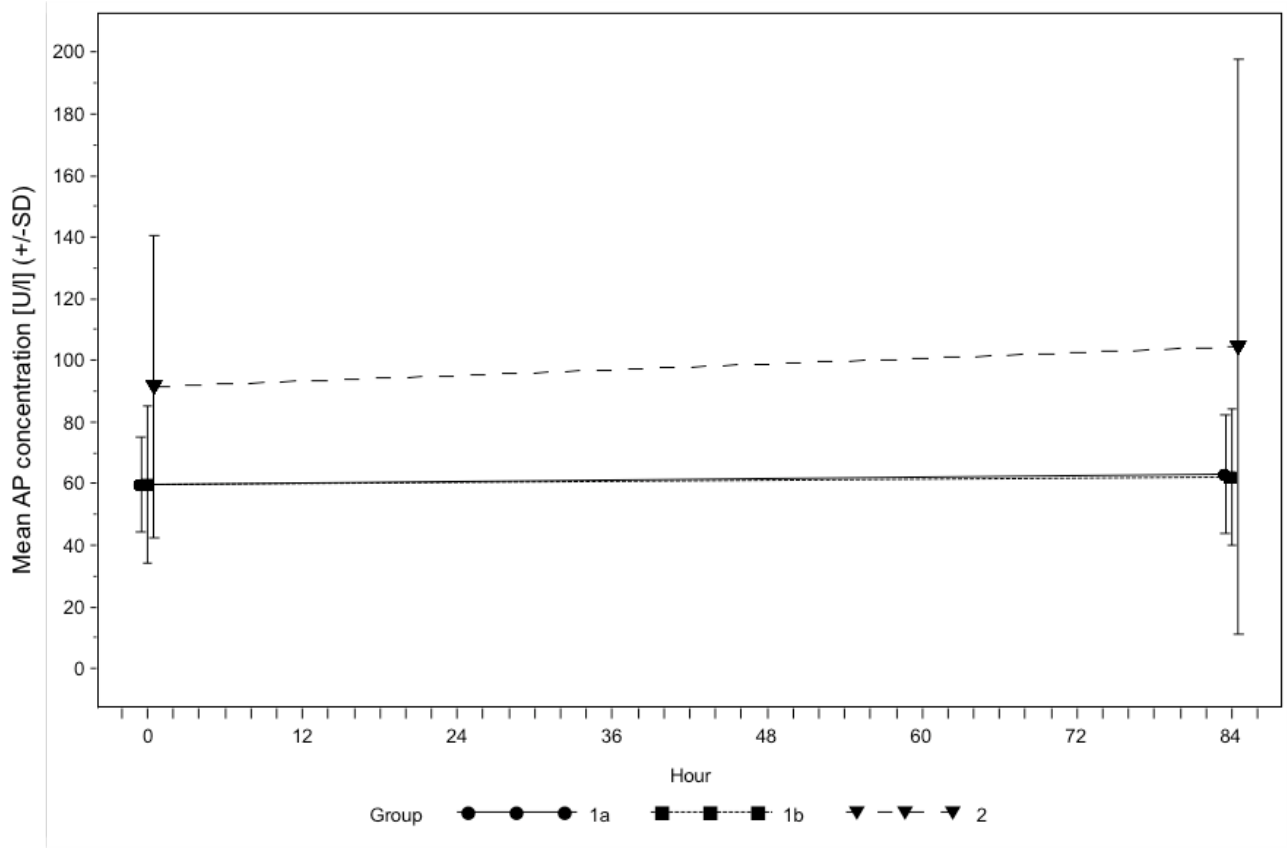

Fig. 45. Time course of mean alkaline phosphatase (AP) level [U/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

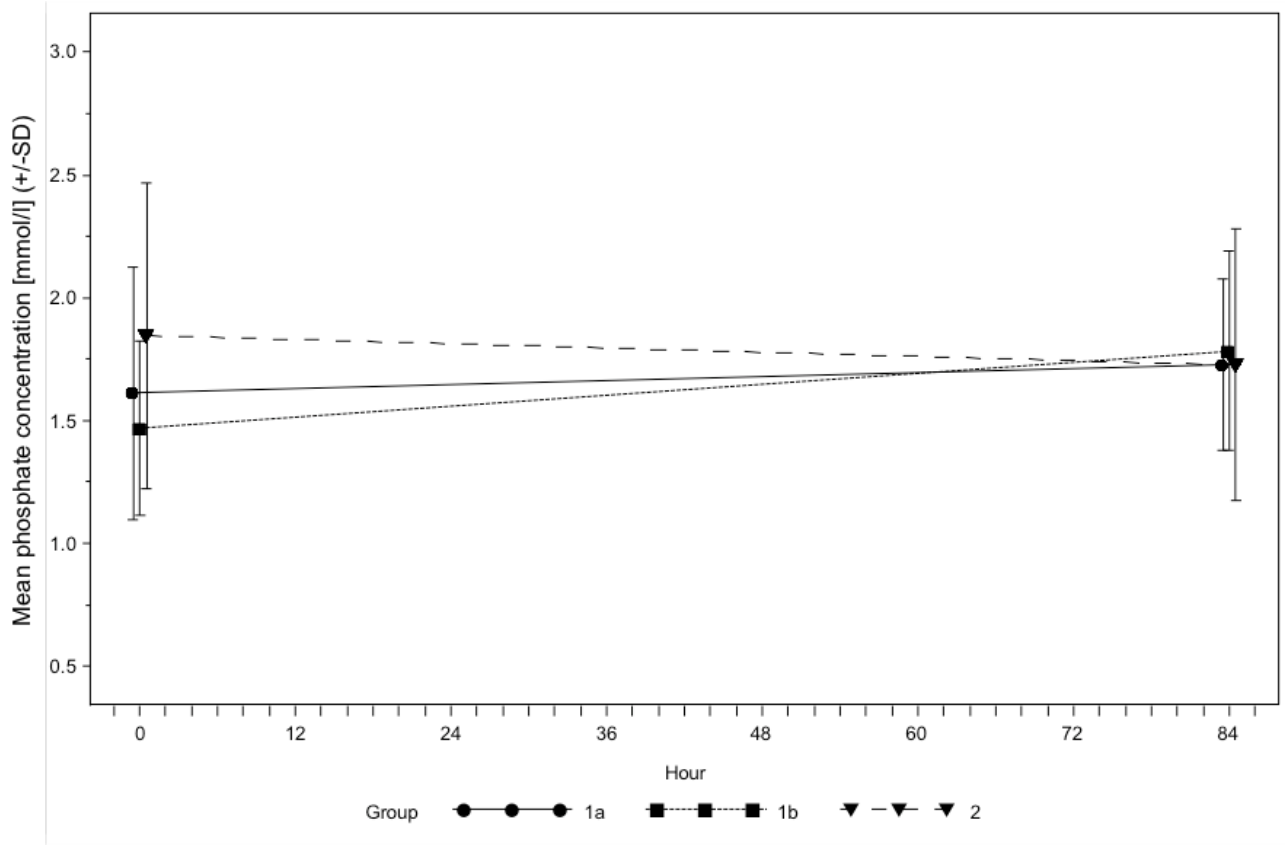

Fig. 46. Time course of mean phosphate concentrations [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

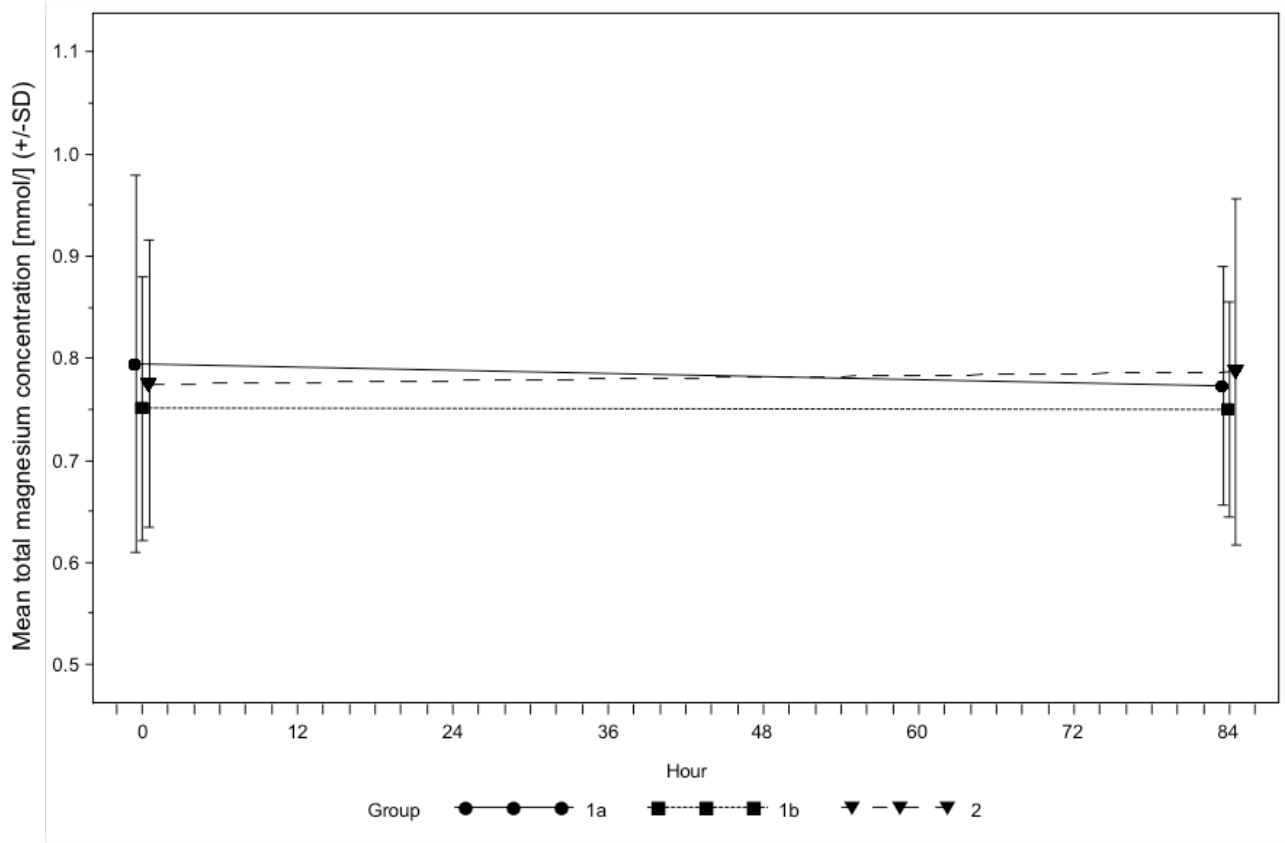

Fig. 47. Time course of mean concentrations of magnesium total [mmol/l ± SD] in cows with hypokalemia. Results are presented of blood samplings before (0 hours) and 84 hours after bolus administration (Group 1a, Group 2) and sham administration (Group 1b).

12 LIST OF PUBLICATIONS

Amiya Olany; Kerstin Müller (2013): Hypokaliämie: Auftreten und klinisches Bild bei Milchkühen in der Frühlaktation. Abstract, DVG-Vet-Congress, Berlin, Germany, 6.-10. November 2013.

Amiya Olany, Kerstin Müller (2013): Hypokalemia: Occurrence and clinical signs in early lactation dairy cows. Abstract, 8th PhD-Symposium / DRS presentation seminar (Doktorandensymposium), Freie Universität Berlin, Germany, 15. July 2013.

Julia Plöntzke, Mascha Berg, **Amiya Olany**, Sabine Leonhard-Marek, Kerstin Elisabeth Müller, Susanna Röblitz (2013): Modeling potassium balance in dairy cows, ZIB-Report (13-09)

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14 DECLARATION OF INDEPENDENCE

Hiermit bestätige ich, Amiya Ricarda Wilhelm-Olany, dass ich die vorliegende Dissertation selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 29. November 2019

Amiya Ricarda Wilhelm-Olany