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Master's Thesis of Veterinary Medicine

Subclinical Ketosis in Dairy Herds

An Evaluation of Four Diagnostic Cowside Methods for Detection of Ketosis

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Title:	Subclinical Ketosis in Dairy Herds
Subtitle:	An Evaluation of Four Diagnostic Cowside Methods for Detection of Ketosis
Topic description:	Subclinical ketosis in early lactation dairy cows is a common problem in many countries, including Denmark. An investigation of agreement between frequently used cowside tests for ketosis screening postpartum, and an evaluation of the effect of results on cow and herd level was made. Furthermore, alternative sampling strategies for ketosis screening was investigated.
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Preface

This thesis concludes my Master in Veterinary Medicine at the Faculty of Health and Medical Science, University of Copenhagen. It is a result of my great interest in the dairy cattle production and wishes of gaining practical veterinary experience in the work at this field. This study has been a major contributing factor to my further way of thinking in the work for dairy cattle purposes.
The project was conducted from September 2019 to February 2020. Data collection was carried out in 14 dairy herds in the Middle-Western Jutland, Denmark. Participating farmers have all signed a declaration of consent which allowed data to be collected and investigated in the current thesis.

I would like to thank my advisor Dorte Bay Lastein ((Adjunct), DVM, Ph.D.) for always being positive, optimistic and helpful. Whenever I experienced bumps on the road, she guided me in the right direction and she made it possible for me to work highly independent through great guidance. I would also like to thank my co-advisor Jörg Matthias Dehn Enemark ((Affiliated Professor), DVM, Ph.D., DIP ECBHM) for always being available with inspiration and help in the understanding of the subject of this thesis and for helping me with contacts for the sponsorships.

I direct a great thanks to Ole Rasmussen (DVM) and his coworkers for the enormous help and inspiration, when I was taught how to collect data in the study. For that, I am truly grateful.
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I would like to send my thanks to all the farmers for their participation in the study; for letting me disturb them in their daily work and for always making me feel welcome on their herds.

There are several companies that I would like to acknowledge for their sponsorships in this study: Kruuse A/S for sponsoring materials (examination gloves, syringes, needles, disposable aprons and rectal gel), Elanco Animal Health for sponsoring KetoTest and 2 BHB-check plus devices, Ascensia Diabetes Care Denmark Aps for sponsoring Ketostix, VikShop (Viking Denmark) for sponsoring electrochemical strips for the BHB-check device and E-vet A/S for sponsoring disposable catheters.

Finally, I would to say thank you to my beloved family, amazing friends and my dearest Simon for their endless support and help throughout this project.

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Abstract

Aims: This thesis consists of a **pilot study**, **study 1** (primary) and **study 2** (secondary). The aim of study 1 was, via practiced methods in the pilot study, to investigate agreement between 4 diagnostic cowside methods for the detection of subclinical ketosis (SCK) postpartum. The aim of study 2 was to investigate alternative sampling strategies for ketosis screening. The dry period was included.

Method: Study1: Data was collected from 488 fresh cows distributed in 14 dairy herds localized in the Middle -and Western Jutland, Denmark. The four cowside diagnostic methods included ketosis screening in: freshly sampled milk (T_{milk}) using KetoTest (Elanco Animal Health); manipulated urine (U_{man}) and catheterized urine (U_{cat}) using Ketostix (Ascensia Diabetes Care), and blood collected in the coccygeal vein (Bl_{orig}) using the BHB-check device (PortaCheck®). SCK was defined as ≥1.2 mmol/L blood β-hydroxybutyric acid (BHBA). Agreement between the four tests was evaluated with i.a. Sensitivity (Se) and Specificity (Sp), Weighted kappa (κ (*w*)), and Coverage probability (CP). Challenges with the accuracy of the BHB-check were experienced. Therefore, a 'correction factor' was calculated, which was used as gold standard (Bl_{corr}).

Study2: Data was collected from 10 dry cows in one dairy herd, localized in the Middle -and Western Jutland, Denmark. Blood BHBA was measured three times a week with the BHB-check device, two weeks prepartum and two weeks postpartum. BHBA variation in the transition period was evaluated with the risk of missing SCK cases depending on screening strategy. The relative risk (RR) for developing SCK postpartum if blood BHBA was increased prepartum (≥0.7 mmol/L), was calculated.

Results: **Study1**: The overall SCK prevalence measured in blood was 50.6% and 9.9% in non-corrected and corrected measurements, respectively. Both U_{man} and U_{cat} showed the best combined Se and Sp using a cutoff of ≥0.5 mmol/L (U_{man}: Se: 0.58, Sp: 0.82, U_{cat}: Se: 0.58, Sp: 0.84). The highest combined Se and Sp in milk was seen using a cutoff of ≥50 µmol/L (Se: 0.42, Sp: 0.85). The agreement between manipulated and catheterized urine was high (κ(*w*):0.863, CP:0.952). **Study2**: A great variation in BHBA in the transition period was experienced. RR was 1.11 but was not significant.

Conclusion: **Study1**: Great variation of SCK prevalence depending on test method was seen. Overall, both urine tests had higher Se than the milk test. There was high agreement between manipulated and catheterized urine. **Study2**: BHBA variation in the transition period yielded a risk of missing SCK cases if screening is only done once. No significant RR between increased BHBA prepartum and SCK postpartum was found. Due to the challenges with the handheld device all results must be read with precaution. It is important to ensure accuracy of used diagnostic tools on a regularly basis.

Resumé

Formål: Dette specialeprojekt består af et **pilotstudie**, **studie 1** (primær) og **studie 2** (sekundær). Formålet med studie 1 var, via øvelse i pilotstudiet, at undersøge overensstemmelse mellem fire 'cowside' testmetoder, til påvisning af subklinisk ketose (SKK) efter kælvning. Formålet med studie 2 var at undersøge alternative strategier for ketose screening. Goldperioden blev inddraget.

Metode: Studie1: Data blev indsamlet fra 488 nykælvere fordelt på 14 besætninger lokaliseret i Midt-og Vestjylland, Danmark. De fire 'cowside' metoder indebar ketose-screening på: frisk opsamlet mælk (T_{milk}) med KetoTest (Elanco Animal Health); manipuleret (U_{man}) -og kateter urin (U_{cat}) med Ketostix (Ascensia Diabetes Care) samt fuldblod opsamlet via halevenen (Bl_{orig}) med BHB-check (PortaCheck®). SKK blev defineret som β-hydroxybutyrate (BHBA) ≥1,2 mmol/L i fuldblod. Overensstemmelse mellem de fire tests blev vurderet ud fra bl.a. Vægtet Kappa og Dækningsgrad samt Sensitivitet (Se) og Specificitet (Sp). Der var udfordringer med nøjagtigheden af BHB-check. Derfor blev en korrektionsfaktor beregnet som erstatning for 'gold standard' (Bl_{corr}).
Studie2: Data blev indsamlet fra 10 goldkøer på én besætning lokaliseret i Midt-og Vestjylland, Danmark. Blod BHBA blev målt 3 gange ugentligt med BHB-check apparatet, 2 uger før -og 2 uger efter kælvning. Blod BHBA variationen i overgangsperioden blev vurderet i forhold til, at overse et SKK tilfælde afhængig af screenings strategi. Den relative risiko (RR) for at udvikle SKK efter kælvning, hvis blod BHBA var øget før kælvning(≥0,7 mmol/L), blev udregnet.

Resultater: **Studie1**: Den overordnede SKK prævalens målt på blod var 50,6% og 9,9% ved hhv. ikke-korrigerede og korrigerede målinger. Både U_{man} og U_{cat} havde den bedste kombinerede Se og Sp med et cutoff på ≥0,5 mmol/L. (U_{man}: 0,58/0,82 U_{cat}: 0,58/0,84). Den højeste kombinerede Se og Sp i mælk, blev opnået ved et cutoff på ≥50 µmol/L (0,42/0,85). Der var høj overensstemmelse mellem manipuleret -og kateterurin (vægtet kappa: 0,863, dækningsgrad: 0,952). **Studie2**: Der blev fundet stor variation i blod BHBA i overgangsperioden. RR var 1,11, men var ikke signifikant.

Konklusion: Studie1: Der sås stor variation af SCK prævalens afhængig af test metode. Overordnet havde begge test på urin højere Se end testen på mælk. Der var høj overensstemmelse mellem screening på manipuleret -og kateterurin. **Studie2**: Blod BHBA variation i overgangsperioden medførte risiko for, at overse SKK tilfælde, hvis man kun screener én gang. Der blev ikke fundet nogen signifikant RR mellem øget blod BHBA før kælvning og udvikling af SKK efter kælvning.
Udfordringerne med det håndholdte apparat gjorde, at alle resultater skal tages med forbehold. Det er yderst vigtigt, at man kvalitetssikrer sine diagnostiske redskaber konsekvent og regelmæssigt.

Abbreviations

Ac: acetone

AcAc: acetoacetate

BCS: body condition score

BHBA: β-hydroxybutyric acid

Bl_{corr}: corrected BHBA measurements (test 4)

Bl_{orig}: original BHBA measurements (test 4)

CI: confidence interval

CK: clinical ketosis

CMT: California Mastitis Test

CP: coverage probability

CPT I: carnitine palmityl transferase I

d: day

D1: Device 1

D2: Device 2

DA: displaced abomasum

DIM: days in milk

DMI: dry matter intake

ECM: energy corrected milk

FPR: fat-to-protein-ratio

FSP-Neo: Freestyle Precision Neo

HHMAS: Herd Health Management Advisory Service

HK: hyperketonemia

id: identification

LCI: lower confidence interval

NEB: negative energy balance

NEFA: non-esterified fatty acids

NTB: nitrotetrazolium blue

pp: postpartum

RR: relative risk

s: seconds

SCC: somatic cell count

SCK: subclinical ketosis

Se: sensitivity

Sp: specificity

TCA: tricarboxylic acid

TG: triglycerides

T_{milk}: measurement in milk (test 1)

TMR: total mixed ration

U_{cat}: catheterized urine (test 3)

UCI: upper confidence interval

U_{man}: manipulated urine (test 2)

VFA: volatile fatty acids

VLDL: Very Low Density Lipoprotein

vs.: versus

1. Introduction

Ketosis is a common carbohydrate and fat metabolism disorder in dairy cattle. Subclinical ketosis (SCK) is defined as elevated ketone bodies in blood (hyperketonemia(HK)), urine, milk and other body fluids *without* clinical signs. Clinical ketosis (CK) is defined as elevated ketone bodies in the various body fluids *with* clinical signs.[1] Clinical signs of ketosis include inappetence, dry hard feces, a sudden decrease in milk production and a rapid loss of body condition score (BCS).[2] The major ketone bodies are β-hydroxybuturic acid (BHBA), acetoacetate (AcAc) and acetone (Ac). Definition of ketosis according to blood BHBA concentration is seen in table 1.[1,3] Dairy cows are mainly in risk of developing ketosis during the first two months of lactation due to increasing milk production and subsequent fat mobilization.[2] The pathogenesis involves an imbalance between the energy demand of the cow for milking and the actual energy intake (referred to as negative energy balance (NEB)).[4]

Table 1. Definition of ketosis according to blood BHBA concentration.

Normal	SCK	CK
< 1.0 mmol/L blood BHBA	≥1.2-1.4 mmol/L blood BHBA	>2.9 mmol/L blood BHBA

The reported worldwide prevalence of SCK was reviewed by Tatone (2016) to range from 8.5% to 58.8%, based on different diagnostic approaches and thresholds in the respective studies.[5] Both peak prevalence of SCK and peak incidence risk of SCK occur at 5 days in milk (DIM).[6] Krogh et al. (2011) found a SCK prevalence among Danish dairy cows to be between 10% and 12% depending on diagnostic method.[7] Ingvartsen reviewed in 2006, that CK only occurs in approx. 1% to 5% of the Danish dairy cows.[8] The accurate current CK prevalence in Denmark is not known.

All periparturient cows undergo a period of NEB. Therefore, the ability of the cow to adapt to NEB and avoid developing ketosis is dependent on several herd-level and cow-level risk factors.[5,9] The most influential herd-level risk factors for ketosis include feeding-management, herd size, season of calving and dry cow management.[10–13] Cow-level risk factors for ketosis include breed, dystocias, increased parity, BCS loss during the dry period and BCS of ≥3.5 at calving.[14–18]

Losses caused by undiagnosed SCK exceed losses by CK.[19] A case of CK has been estimated to cost between approx. 546DKK and approx. 1584DKK depending on parity, treatment and downstream consequences.[20,21] A single case of SCK has been estimated to cost approx. 797 DKK,[22] although approx. 1920DKK when accounting for all the downstream consequences associated with SCK.[23] A threshold for HK based on blood or serum BHBA between 1.2-1.4 mmol BHBA/L has been suggested by leading scientists based on these consequences.[3,6,23,24] SCK is associated with increased risk of displaced abomasum (DA),[25,26] increased risk of CK and a greater risk of metritis within the first or second week postpartum (pp).[26,27] Furthermore a risk of having cystic ovaries[28] and increased risk of retained placenta has been associated with SCK.[27,29,30] An increased blood BHBA concentration has a negative impact on the immune system.[31] Cows with SCK are more likely to be culled than non-ketotic cows in the first 30 DIM.[6] SCK in the first two weeks of lactation reduces the chance of pregnancy a first insemination.[24] SCK in the first week pp results in a milk loss of 1.88 kg/cow/day in the first 30 DIM and a 305-day milk loss of 333 kg/cow (serum BHBA ≥1.8 mmol/L) in the first week pp compared to non-ketotic cows.[26]

Multiple biochemical tests are available to diagnose ketosis. Accurate quantitative measurements of the ketone bodies in biological fluids are available, but these tests are also time-consuming and expensive.[32,33] Alternatively, several commercial cowside tests are available. The advantages of these tests are being inexpensive and gives results immediately. The disadvantages of the cowside tests are a lower sensitivity (Se) and specificity (Sp) compared to a gold standard.[1,34,35] In a herd, the optimum is to diagnose all positive cows with the lowest amount of money and time spend, which may increase the risk of missing cases of SCK. McArt et al. (2012) discovered a higher risk of developing DA, to be culled and producing less milk when SCK (≥1.2 mmol/L serum BHBA) was diagnosed from 3 to 5 days pp than 8 days pp or later.[6] This suggest that screening of SCK should be obtained in the first week pp. A prepartum blood BHBA concentration of ≥0.6 mmol/L have been associated with a risk of SCK pp.[36] This suggest that monitoring of SCK should include the dry period. Other methods for monitoring of ketosis include rumination time around calving,[37] loss of BCS in the dry period,[18] milk fat-to-protein-ratio (FPR),[7,38,39] and biomarkers in milk to detect physiological imbalance in dairy cattle.[40] Measurements of BHBA in milk has been included in the RYK-Livestock Registration and Milk Recording system[41] in Denmark since October 2013 to monitor of whether the herd experience SCK among cows in early lactation or not.[42]

Since July 2010, Herd Health Management Advisory Service (HHMAS) has been mandatory in Danish dairy herds with ≥100 cows.[43] The optional module 2 agreement allows the farmer to initiate treatment of selected conditions from which a ‘herd diagnosis’ has been established, together with a relative high frequency of visits from the veterinarian (‘routine-examinations visits’). The visits should include clinical examinations of fresh cows (5-21d pp) with 7d/14d/21d interval according to the herd size.[44] The examinations could include i.a. an evaluation of BCS, uterus, and a screening of ketosis. The examinations are important to obtain an evaluation of ketosis prevalence on herd level, especially with cows without clinical signs. At present, the legislation has been changed. Frequency of veterinary visits on farms is decreased and fresh cows screenings are not compulsory.[45] However, a large proportion of the Danish cattle practitioners continue to perform routine screenings of fresh cows, though the exact extend and validity of the data is unknown at present.[46] Cowside measurements of BHBA in milk and AcAc in urine probably are the most common tests used in Denmark and the screening of ketosis is supposedly made during the 'routine-examination-visits' even though no formal data is available.[7,46] Despite practical advantages of these tests, their usage do possess some disadvantages, particularly that the Se and Sp tends to decrease with test method as follow: blood>urine>milk,[5,34] which increases the risk of missing true positive cases (both clinical and subclinical). This is questioning the size of agreement between cowside tests and their different sampling technics (e.g. blood sampling, manipulated urine (stimulated micturition), urinary catheterization) used in different biological fluids for ketosis screening pp under Danish conditions. A median length of a SCK case was demonstrated to be 5 days, which means that a single screening of ketosis involves a risk of missing cases of SCK.[6] Therefore, alternative diagnostic and sampling strategies may be beneficial.

1.1. Objectives

SCK in early lactation dairy cows is a common problem in many countries, including Denmark. Screening of fresh cows is frequently done using semiquantitative cowside urine and milk tests once between 5-21 DIM though these tests have lower Se. After developing practical, time efficient, and valid sampling technics (stimulated micturition, urinary catheterization, and using a handheld blood device) I will investigate the agreement between frequently used cowside tests for ketosis screening pp and evaluate the effect of results on cow and herd-level. If ketosis starts to develop in the close-up dry period and cows may develop SCK and return to normal within 5 days, the risk of missing cases of SCK increases, if these considerations are not taken into account. Therefore, I will investigate alternative sampling strategies for ketosis screening with an inclusion of the dry period.

1.2. Hypotheses

- Semiquantitative tests underestimates the prevalence of SCK compared to quantitative tests
- There is a high agreement between diagnostic tests performed on vaginal stimulated micturition and catheterized urine in the diagnosis of SCK
- Variation in periparturient blood BHBA concentrations results in missing cases of SCK pp
- Elevated blood BHBA in the dry period is associated with SCK in early lactation

2. Literature review

To fully understand the complexity of ketosis and to understand the background of the diagnostic methods, the pathophysiology of ketosis and common diagnostic methods has to be described.

2.1. Ketogenesis

Ruminants have developed specific adaptational physiological processes to attempt to cope with the NEB situation in the periparturient period.[4] In ruminants all dietary carbohydrate are fermented in the forestomach by the ruminal microbes, resulting in the production of the volatile free acids (VFAs) of which the main are; *acetic acid, propionic acid* and *butyric acid* (often referred to as their dissociated ions: acetate, propionate and butyrate, respectively). VFAs are mainly absorbed through the ruminal wall by passive diffusion and transported to the liver via the portal vein.[47] The rate of intraruminal VFA absorption is depended of ruminal pH and thus the degree of dissociation of VFAs.[48] VFAs are the main energy source in the ruminant species.[47] The approximate average proportion of the VFAs in the rumen are: acetic acid, 63; propionic acid, 23; butyric acid, 12, but varies highly according to diet.[49] Milk production requires large amount of carbohydrate in the form of glucose for lactose synthesis. In ruminants, this requirement is accomplished by the process *gluconeogenesis*. Propionate is a major substrate for this process. Other precursors include circulating glucogenic amino acids and glycerol.[50] Acetate and butyrate cannot work as precursor for glucose (nor later for lactose).[4] Instead, acetate enter the tricarboxylic acid (TCA) cycle as acetyl CoA where it is utilized for energy production in peripheral tissues and is a major precursor for fatty acid synthesis during anabolic periods.[47] At least 50% of butyrate is metabolized in the rumen epithelium to ketones (primarily BHBA).[50,51] The amount of butyrate that enters the liver is oxidized in the TCA cycle or may be used as carbon substrate for lipid production.[8,50,51] Therefore, some degree of ketone body formation is a result of normal digestion in the ruminants. Cows fed silages with high concentrations of butyrate will have an elevated level of BHBA in the blood (*butyric acid silage ketosis=secondary ketosis*).[52]

In early lactation, the production of lactose rises dramatically, which increases the demand for glucose. In turns, this speeds up gluconeogenesis and decreases the glucose consumption in most tissues but the udder. In spite of these compensatory efforts, blood glucose concentration drops, especially in older dairy cows due to a high milk production and the subsequent high lactose demand.[53] During NEB, the dairy cow seeks to meet the increasing energy demands by mobilizing stored energy from adipose tissue. Adipose tissue consist of triglycerides (TG), consisting of three

long-chain fatty acids esterified to a glycerol molecule. The ester-bond will constantly be cleaved and reformed within the adipocytes by processes known as *lipolysis* and *lipogenesis*, respectively (figure 1). Lipolysis results in the release of non-esterified fatty acids (NEFAs) which, bound to albumin together with glycerol, are transported in the blood and absorbed by the liver.[4] NEFAs can participate in the fuel homeostasis where they may contribute as energy in peripheral tissues or be absorbed by the liver and channeled into different pathways.[51] The lipolysis–lipogenesis balance is regulated by the concentration of glucose and insulin. During NEB these concentrations are diminished. This results in a dramatically increase in NEFA release from adipose tissue.[4]
NEFA can enter three different pathways once inside the liver (figure 1):[8]

1. Completely oxidized to CO_2 thereby providing energy via the TCA cycle
2. Partially oxidized for the production of ketone bodies
3. Exported from the liver as Very Low Density Lipoproteins (VLDL) and subsequently reconverted into TGs

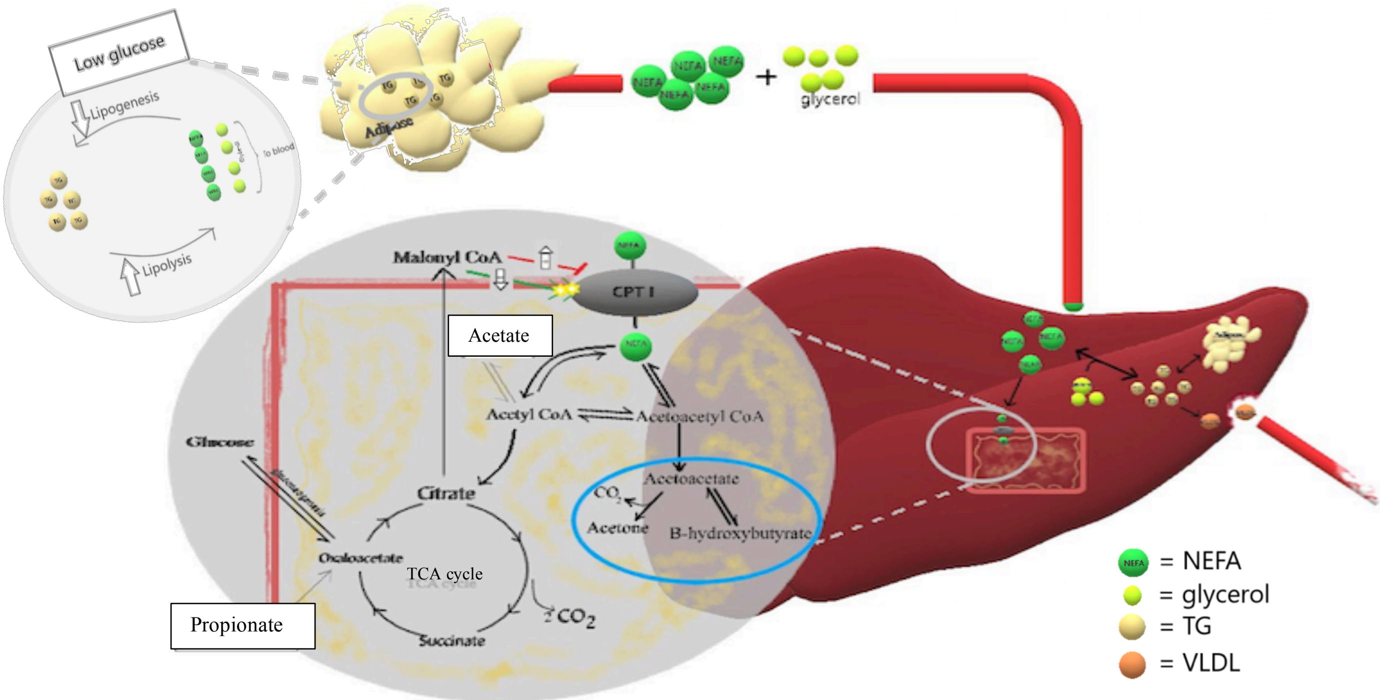


Figure 1. During negative energy balance, blood glucose drops. This causes increased lipolysis and release of non-esterified fatty acids (NEFA) to the blood. In the liver, NEFA either go to the mitochondria and get completely oxidized to CO_2 or become partially oxidized to produce ketone bodies (blue circle). Otherwise, it is exported as very low lipo-proteins (VLDL) with a subsequent reconversion to triglycerides (TG) and deposit inside the liver. Figure by: M. Houe

Inside the liver, further metabolic events of NEFAs are either mitochondrial or cytosolic. When blood glucose concentration is adequate, the glucose transport into the TCA cycle is uninhibited. This results in an accumulation of TCA cycle intermediates such as citrate. This surplus of citrate is transported to the cytosol where it converts to *malonyl CoA*, the first metabolite in the synthesis of fatty acids.[4] The enzyme *carnitine palmityl transferase I* (CPT I) is located in the mitochondrial wall. It is necessary for the transport of NEFAs into the mitochondria for synthesis of ketone bodies (figure 1). The activity of CPT I is dependent on the concentration of malonyl CoA.[4] During NEB, when the cow faces a high glucagon/insulin ratio and almost all glucose is being used to lactose production, only small amounts of glucose will flow into the TCA cycle. Therefore, limited amounts of citrate will leave the mitochondria for fatty acid synthesis.[4,51] Hence, the concentration of malonyl CoA will lower. This results in high activity of CPT I and thus, high-level transport of NEFAs to the intramitochondrial compartment. Once inside the mitochondria, NEFAs can either become completely oxidized to acetyl-CoA for energy production in the TCA cycle or get partially oxidized for ketone body synthesis.[4,5,54] However, during massive lipolysis, fatty acids are rapidly entering the mitochondria. When the concentration of oxaloacetate (an intermediate in the TCA cycle) is low due to the consumption of this substrate in gluconeogenesis, this results in more acetyl CoA available than the need of the TCA cycle.[8] Then, the fatty acids will only be partially oxidized.[55,56] Instead, Acetyl CoA is metabolized to acetoacetyl CoA, a precursor of one of the three major ketone bodies, AcAc.[54] The proportion of NEFAs that do not get completely oxidized or enter the ketogenesis pathway in the mitochondria is re-esterified with glycerol in the cytosol to form TG (figure 1). Export of TG for utilization in other tissues requires synthesis of VLDLs.[4] The capacity of the liver to synthetize the protein components of VLDLs, is exceeded by its capacity to form TGs.[51] During periods of excessive fat mobilization where NEFA uptake in the liver is pronounced, the liver is not capable of exporting all the TGs, resulting in an accumulation of fat inside the liver (*fatty liver*). Depending on the level of NEB in the periparturient period, a physiological to pathological degree of fatty liver will develop in the fresh cows.[8] In summary, this massive mobilization of TGs from fat tissues, high-level entering of NEFAs into hepatocytic mitochondria and subsequent formation of ketone bodies, result in an increased level of circulating ketone bodies in dairy cows in early lactation. This is referred to as the metabolic state *ketosis*.

The three major ketone bodies are shown in figure 1 (blue circle). BHBA and Ac are derivates of AcAc. AcAc is by itself an unstable compound so after entering the cytosol, much of it will rapidly convert to either BHBA under the influence of the enzyme β-hydroxybutyrate dehydrogenase (reversible) or break down via a nonenzymatically process to form Ac and CO_2 (irreversible).[54] Ac is volatile which causes the characteristic odor of the breath from ketotic cows.[51,54] In the non-ketotic cow, BHBA represent the main ketone body (BHBA:AcAc ratio >51).[57] However, during ketosis the AcAc production and secretion increases which declines the BHBA:AcAc-ratio to 5.3.[57]

Ketone bodies are distributed to peripheral tissues for energy production. The tissues utilize ketone bodies by converting BHBA to AcAc which in turn is converted to acetoacetyl CoA and subsequently acetyl CoA.[51] Acetyl CoA can be oxidized in the TCA cycle for energy production or can be used for the synthesis of fat, a process that particular takes place in the mammary gland resulting in an increased milk fat percentage and FPR during episodes of ketosis.[51,54,58] In the cow, excess ketone bodies undergo mammary and renal excretion. Ac can also be exhaled.[51,54]

2.2. Diagnostic measurement of ketone bodies

Both blood, urine and milk can be used for diagnosis of ketosis.[32] Laboratory measurement of BHBA is considered as the gold standard, due to the fact that BHBA is the most stable ketone body in blood.[32,59] Since laboratory measurements are both costly and delay the diagnostic process, several cowside semiquantitative diagnostic methods have been made available.[60] Cowside tests show a lower Se and Sp compared to laboratory tests, which should be taken into account when interpreting the results. Furthermore, other conditions may have an impact on the ketosis screening e.g. diurnal variation of ketone body levels, site of blood sampling, method used for urine collection, somatic cell count (SCC) in milk, and the fact that there are substantial differences between ketone bodies in blood, urine and milk. One study discovered that the BHBA concentration in milk had a significant diurnal variation with the highest concentration of BHBA between milking and lowest concentration at milking.[61] Both AcAc and BHBA may be used when measuring ketone body levels in blood. However, because of the instability of AcAc, BHBA is preferred when screening for ketosis in field studies.[5,32] A correlation coefficient of 0.66 between blood BHBA and milk BHBA has been found.[62] Urinary excretion of AcAc (+Ac) follows a log-log pattern when compared to ketone body levels present in the blood.[63] During SCK, ketone body levels in urine may exceed the blood ketone concentration by four times.[64]

2.3. Ketone body measurement in practice

BHBA measurement in blood is the most sensitive cowside test for diagnosing SCK and CK in dairy cattle.[5,34] However Jeppesen R. (2005) showed that the participants of her questionnaire (approx. 10% of the total Danish cattle practitioners) used semiquantitative diagnostic methods on either urine or milk[65] Due to the lower Se of tests used on these body fluids, an unknown number of SCK cases may not be diagnosed. Urine sampling for ketone body measurements may either be done by perineal or vaginal stimulation or by catheterization. Weakness of these methods are that stimulated micturition may be time consuming and there is an inherited risk of present lochial discharge, interfering with the color change on the strip when using vaginal stimulated micturition. On the other hand, catheterization requires a high level of hygiene and skills.

Cowside diagnosis of SCK is important to optimize herd, feeding and production management and to prevent outbreak of CK.[66] Several commercial products are available for testing ketone body levels in milk, urine and blood but they are more or less based on the same chemical or electrochemical reaction. In the current thesis, three commercial products are investigated; the **BHB-check device** (PortaCheck® Inc., 1 Whittendale Drive Suite E Moorestown NJ 08057, USA), **Ketostix** (Ascensia Diabetes Care Denmark Aps., Arne Jacobsen Alle 7, 2300 Copenhagen S, DK), **KetoTest** (Elanco Animal Health Netherland, Van Deventerlaan 31 3528AG Utrecht).

2.3.1. BHB-check

BHB-check is a small handheld meter (weight: 48.5g, dimensions: 8x6x2 cm) designed to measure ketone body concentrations in bovine blood. BHB-check measures BHBA concentrations in whole blood quantitively (0.1 – 8.0 mmol/L). Electrochemical strips are used for the measurement of BHBA. The strips are stored in packets of 10, 25 or 50 strips. 0.7 µL whole blood must be applied in the chamber of the electrochemical strip. The strip contains β-hydroxybutyrate dehydrogenase and some non-reactive chemicals. BHBA in the blood reacts with the enzyme and generates an electric current which is measured by the BHB-check device and, within 5 seconds(s), is converted into BHBA concentration (mmol/L) which is displayed on the screen. The recommended temperature for optimal operating conditions is 10°C to 40°C and for the strip storage 2°C to 30°C. BHB-check is only capable of measuring BHBA, although the BHB-check plus is capable of measuring both blood glucose and BHBA concentrations.[67] The BHB-check plus device was used in the current thesis. Using a cutoff value of ≥1.2 mmol/L blood BHBA for the diagnosis of SCK, BHB-check has been shown to have an overall Se of 91% and Sp of 93%.[68] The test strip costs approx. 14.5 DKK/strip and the device costs 306DKK (incl. 10 strips) (taxes excluded).[69]

2.3.2. Ketostix

Ketostix is an inexpensive (approx. 2 DKK/strip) and easy to use test strip which measure the concentration of AcAc (and to a lesser degree Ac) in urine. The test was developed for human use, but has since been adopted by cattle practitioners.[60] The test strip contains glycine buffer and sodium nitroprusside. When AcAc reacts with nitroprusside a color change occurs, ranging from buff-pink to maroon, depending on the concentration of ketone bodies in the sample. When the urine sample contains at least 0.5 mmol/L AcAc per liter, the test strip provides a positive reaction.

The color chart offers readings for ketone concentrations of 0.5, 1.5, 4, 8 and 16 mmol/L, respectively.[70] According to the manufacturers recommendation, strips must be read at 15 s after sampling.[71] Jeppesen (2005) showed that the time span from initiating to reading the strip influences the Se and Sp of Ketostix.[72] Using a cutoff of "small" (1.420 mmol/L) a Se of 78% and a Sp of 96% of the Ketostix has been found, when defining SCK as ≥1.400 mmol/L blood BHBA.[34]

2.3.3. KetoTest

KetoTest is another easy to use test strip for diagnosis of ketosis in dairy cattle. KetoTest measures the concentration of BHBA in freshly sampled milk. When milk is applied on the test strip, BHBA is converted by the β-hydroxybutyrate dehydrogenase enzyme into AcAc. This reaction produce NADH from NAD+. The produced NADH reduces nitrotetrazolium blue (NTB) to formazan which has a purple color. According to the change in color, the BHBA concentration can be estimated. The color chart gives readings for 50, 100, 200, 500 and 1000 µmol/L. The test must be read at 60 s. The strips should be stored at 2°C to 8°C and brought to room temperature before use. The costs of KetoTest is approx. 12DKK/strip.[73] Jeppesen R. (2005) showed that high levels of SCC in the milk may give false positive results when using a semiquantitative test.[72] With a cutoff value of ≥100 µmol/L KetoTest has been shown to have a Se of 73% and Sp of 96%.[34]

2.4. Monitoring ketosis at herd and cow level

Monitoring ketosis prevalence is important to detect changes in disease occurrence and management pattern.[5] According to Oetzel (2007), diagnostic measurement of ketosis in a herd-based testing setup, requires a subsample of ≥12 animals that are at risk for ketosis (5 to 50 DIM), followed by an evaluation of the proportion of cows above the cutpoint.[52] If the proportion exceeds the chosen alarm level, a herd should be classified as potential ketosis problem herd. McArt et al., (2012) demonstrated that the median time from the first positive blood BHBA test until the first negative blood test (<1.2 mmol/L) was 5 days.[6] If testing only occurs once pp, the true prevalence of SCK in the herd may be understimated.[6,26] One study investigated important predictors of developing ketosis at any time between 3 to 16 DIM and for a cow to show ketosis at her first BHBA measurement pp (3 to 5 DIM).[14] The cows in the study were individually tested three times a week between 3 to 16 DIM and defined as SCK when blood BHBA concentration was ≥1.2 mmol/L. In consideration of variation in SCK incidence between herds, increased parity and increased BCS were both predictors of SCK development any day between 3 to 16 DIM (=model 1). In consideration of variation of SCK incidence between herds, increased parity, male calf and increased BCS were predictors of SCK between 3 to 5 DIM (=model 2).[14] The study demonstrated that model 2 had higher predictive value than model 1 (87% and 69%, respectively).[14,60] Around 60% of fresh cows develop SCK within 3 to 5 DIM.[6] These cows are furthermore at higher risk of developing the negative downstream consequences compared to cows that develop SCK later.[6] In summary, it can be argued, that ketosis screening in fresh cows the first week pp seems more clinically relevant.

2.5. Transition period

The transition period has been defined as three week prepartum until three week pp based on changes in hormones, energy requirements and metabolism in this period.[74] Placental and fetal glucose demands in late gestation together with mammary gland lactose production in early lactation, lead to an increased glucose utilization during this period.[75] Three weeks before calving, dry matter intake (DMI) drops 30-40%, with 89% of the decrease occurring in the last week of gestation.[76] Further drops in DMI and less time spend feeding have been associated with occurrence of SCK (≥1.0 mmol/L serum BHBA).[77] The increasing energy requirement during the transition period leads to fat mobilization resulting in increased plasma NEFA levels.[74] Plasma NEFAs start increasing two weeks before calving, peaking at calving and may remain high for up to three weeks pp.[5,78,79] This could lead to the assumption that the development of ketosis starts during the close-up dry period. Tatone et. al. (2015) have investigated the association between prepartum blood BHBA concentrations and the risk of developing SCK (≥1.2 mmol/L blood BHBA) in the first week pp. They showed, that using a cutoff value of ≥0.6 mmol/L blood BHBA prepartum, cows above this value were 2.2 times more likely to develop SCK in the first week pp than cows below the cutoff.[36] Furthermore, they recommended ≥0.7 mmol/L blood BHBA as cutoff value, when using a handheld device for monitoring fresh cows for ketosis.[36] Several studies have investigated the relationship between an elevated blood BHBA concentration prepartum and increased risk of disease pp.[80,81] One study showed, that cows with a prepartum blood BHBA concentration of ≥0.8 mmol/L had an increased risk of developing DA within 60 d after calving.[80] Another study showed, that cows with a prepartum blood BHBA concentration of ≥0.7mmol/L in the last week before calving, were at increased risk of culling within 60 DIM.[81] In summary, it can be argued, that ketosis monitoring in a herd should include the close-up dry period.

3. Method and Materials

3.1. Pilot study: Practice of Sampling Technics

The aim of the pilot study was to develop practical, time efficient and valid sampling technics: Vaginal stimulated micturition, urinary catheterization on cows, and use the BHB-check device for cowside testing. This study should support the validity of the data collection in study 1.

The pilot study was conducted during two weeks in September 2019 in a veterinary dairy cattle practice in the South-Western Jutland, Denmark. The visited dairy farms were chosen based on selected veterinarians 'routine-examination visits'. Six cattle practitioners took part in the training of the author. During the first week, the author was taught how to place a urinary catheter, perform stimulated micturition by compression of the vaginal floor and sample blood from the coccygeal vein for the BHB-check device. In the last week, a systematic method for collection of data for study 1 was developed.

The data for study 1 included four tests;

- Test 1 (T_{milk}): Semiquantitative measurement of BHBA in freshly sampled milk using KetoTest.
- Test 2 (U_{man}): Semiquantitative measurement of AcAc in freshly sampled urine by vaginal stimulated micturition using Ketostix.
- Test 3 (U_{cat}): Semiquantitative measurement of AcAc in catheterized urine using Ketostix.
- Test 4 (Bl_{orig}): Quantitative measurement of BHBA in whole blood, sampled in the coccygeal vein, using the BHB-check device.

An outfit was developed which made it possible to wear all the necessary equipment (figure 2). The outfit consisted of a 'home styled' belt with two bags and several pockets; one bag for one packet of KetoTest and two packets of Ketostix and another bag for syringes, needles, the handheld device and the matching electrochemical strips. This would minimize the time spend on the examination of each cow. The disposable catheters were kept in the bag, in the author's boot, making catheters accessible and easy to grab during the examinations. The packets of KetoTest and Ketostix were kept opened during the time spend on the farms in the current study and the test strips from U_{man} and U_{cat} were read simultaneously, which yielded a larger time interval before reading U_{man} than U_{cat}. Both were changed in study 1 (after 2.5 days). The final systematic method is described in further details in section 3.2.2.

Figure 2. The outfit developed for data collection in study 1.

3.2. Study 1: Agreement of Four Cowside Methods for Screening of Ketosis

The aim of study 1 was to investigate agreement between present cowside methods for ketosis screening pp and evaluate the effect of results on cow and herd level.

3.2.1. Herds and animals

The study took place in September 18 until November 14, 2019 in the Middle-Western Jutland, Denmark. The author followed mainly one cattle practitioner on his 'routine examination visits'. In a few days, two other cattle practitioners from the same practice were followed on their visits, to obtain the maximum amount of fresh cows.

Summary information of the 14 herds included in study 1 is showed in table 2.

Table 2. Summary information of the participating herds in study 1.

Herd	Total cows (n)[1]	Breed[2]	Approx. ECM (kg) pr. year[3]	Fresh cows in study 1	Visit interval (d)	Approx. DIM[4]
1	547	HOL	11,600	43	7d	4 - 10
2	338	MIX	12,600	45	7d	4 - 10
3	168	HOL	13,000	32	14d	3 - 14
4	937	JER	11,000	111	7d	4 - 10
5	371	MIX	11,300	8	7d	4 - 10
6	302	HOL	12,500	4	14d	4 - 14
7	526	HOL	12,500	44	7d	4 - 10
8	199	HOL	11,400	8	14d	4 - 21
9	365	HOL	14,400	30	7d	3 - 10
10	408	MIX	11,500	45	14d	4 - 14
11	518	MIX	12,000	81	7d	4 - 10
12	264	HOL	11,000	23	14d	4 - 14
13	378	HOL	12,700	16	7d	4 - 10
14	212	MIX	10,600	10	7d	4 - 11
Total	5,533			500		

[1]January 11 2020, [2]Major proportion of breed. HOL=Holstein cows, JER=Jersey cows, MIX=Mixed breeds. [3]January 2020. ECM=energy corrected milk. [4]DIM for the fresh cows at visits.

3.2.2. Sampling

Data collection included the four test described in section 3.1: T_{milk}, U_{man}, U_{cat}, and Bl_{orig}. Other clinical examinations were performed by the cattle practitioners. These results are not included in the present study. Semi-sterile disposable catheter ("mare-catheter") 25 cm[82] was used for U_{cat}. A 21G needle and 2 ml syringe plus electrochemical test strips for the BHB-check were used in Bl_{orig}. Rectal examination gloves and rectal gel (Bovivet gel[83]) were used for U_{man} and U_{cat}.

Two different BHB-check devices were used during the study period; one device (D1) was used for the first 391 fresh cows, the rest fresh cows were measured using another device (D2). The reason for shift of devices was, that D1 broke after blood entered the insertion site of the device. The same batch number of electrochemical strips were used for all the D1 measurements. When D2 was used, another batch of electrochemical strips was put into service at approx. the same time as the shift in devices. The BHB-check was calibrated, with according electrode every time a new batch of strips was taken into use, according to the manufactures recommendations.[67]

The KetoTest was stored between 2°C to 8°C before the packets was opened for the first time and was afterwards kept at temperature according to the weather. KetoTest and Ketostix packets were kept opened at the first 31 observations as described in section 3.1. In the rest of the study period, the packets were only opened immediately before use. A disposable apron was worn in front of the belt to ensure biosecurity.

The systematic method developed in the pilot study for collection of data in study 1 included:

1. The identification (id) of the cow (ear mark) was registered on a laminated result scheme.
2. 1-3 strips of milk was milked out from one quarter (most frequently from one of the back quarters) and freshly sampled milk was applied directly on the KetoTest strip reaction area (T_{milk}). The strip was placed on a laminated color chart for reading approx. 60 s afterwards (the author was counting the time manually)
3. In the meantime, the vulva was cleaned with a disposable napkin before urine was manipulated out of the urethra. The Ketostix urine strip was directly wetted on the reaction area. The strip was placed on the back of the cow until read approx. 15 s afterwards (U_{man}).
4. During the 15 s, the cow was catheterized (U_{cat}). A maximum of 3 attempts for catheterization was performed. A diagnosis of uterine infection excluded catheterization of the cow.
5. The Ketostix of U_{man} was read after comparison with the laminated color chart. The result of the KetoTest was read simultaneously and a score of 10 to 15 (0, 50, 100, 200, 500 and 1000 µmol/L, respectively) was given. A score of 1 to 6 (0, 0.5, 1.5, 4, 8 and 16 mmol/L, respectively) was given for U_{man}.
6. A Ketostix was wetted directly by the catheterized urine and read approx. 15 s afterwards via comparison with the color chart (U_{cat}). A score of 1 to 6 was given.
7. An electrochemical strip was inserted into the BHB-check device.
8. The ventral one third of the tail was cleaned with a disposable napkin, before 0.1-1 ml fresh whole blood was sampled from the coccygeal vein and immediately transferred to the sample chamber in the electrochemical strip. After 5 s the BHBA concentration (mmol/L) was read.
9. All results were registered on the laminated result scheme. The results were transferred to an excel file in the end of the day. The excel file contains the following information: id of the herd,

id of the cow, parity, results of T_{milk}, U_{man}, U_{cat} and Bl_{orig}, error code (reasons for missing observations), comments and dates. Parity was looked up in www.chr.fvst.dk.

Two videos were made; one video that shows the conduction of T_{milk}, U_{man}, U_{cat}, and Bl_{orig}, and one video that demonstrates the use of the BHB-check device.

3.2.3. Statistical analysis

Data editing and statistical analysis were performed using the computer software RStudio (version 0.99.491- © 2009-2015, RStudio, Inc.) and Microsoft® Excel for Macintosh (version 16.16.17 (191208)). The book by Houe et al. (2004) was used as reference for data editing.[84]

Summary statistics of raw data included observations in herds according to parity (1st, 2nd, ≥3rd), tests (T_{milk}, U_{man}, U_{cat}, Bl_{orig}), number of missing observations, and number of cows with all four (complete) observations. A histogram of error codes as reasons for missing observations in study 1 was performed. Pie charts demonstrating the three major reasons for missing observations according to test method were performed. Subjects with missing values in any of the four tests were deleted. All further analysis were conducted with the complete data set. Appendix A shows both the raw and complete dataset.

Estimation of bias of the BHB-check device

During the study period, the accuracy of the BHB-check was questioned, due to a relatively large proportion of fresh cows with elevated blood BHBA concentrations in which none tested positive by neither T_{milk}, U_{man}, nor U_{cat}. For this reason, an exploratory diagnostic work was done to evaluate the BHB-check, additionally. First, six blood samples were collected in heparin-stabilized blood tubes from six dry cows and transferred to the home practice laboratory. Each blood sample was quantitatively measured by the BHB-check and another handheld device, the FreeStyle Precision Neo (FSP-Neo) (Abbott Diabetes Care Inc., Mississauga, ON, Canada) with matching blood ketone strips. FSP-Neo is developed for human use and may be used for measurement of both blood glucose and blood BHBA concentrations.[85] Two studies have previously showed that the FSP-Neo is an accurate diagnostic method for SCK diagnosis in dairy cattle.[86,87] The six heparin-stabilized whole blood samples were afterwards stored at about 5°C in approx. 18 hours, then transferred to an external laboratory (Idexx Laboratories, Vet Med Labor GmbH, Mörikestr. 28/3, D-71636 Ludwigsburg, Germany) for photometrical evaluation of the BHBA concentration in serum.[88] The results of the six dry cows indicated that the BHB-check was biased as all results were higher than both FSP-Neo and laboratory results. 3 out of 6 blood samples had a hemolysis index (+ to ++++).

FSP-Neo and laboratory results were considered comparable. Therefore, FSP-Neo was considered as 'gold' due to the comparison with the photometrical evaluation performed by the external lab.[89] Subsequently, 19 fresh cows from one of the visits were evaluated with both the BHB-check and FSP-Neo, simultaneously. A plot of raw data demonstrating the distribution of BHBA concentrations from the 19 fresh cows were initially made and showed evidence of systematic bias, since all measurements of the BHB-check were higher than the measurements of the FSP-Neo. One BHB-check device was used in this investigation (D1). The results from the BHB-check and FSP-Neo on the 19 fresh cows and the six dry cows were used to calculate a 'correction factor' for the measurements of Bl_{orig}.

The correction factor analysis was performed in Microsoft Excel in order to demonstrate and quantify a possible bias of the BHB-check. Bland Altman is a graphical analysis based on quantification of agreement between two measurements, thereby demonstrating a bias of one test compared to another. It is constructed by plotting the differences between two tests against the mean of the two tests. The line of equality (=0) implies no difference between the two tests.[90] The bias of the BHB-check was explored by inspiration of the Bland Altman analysis 'plot difference as percentage': method A-method B / mean %;[90] BHB-check replaced method A, FSP-Neo replaced method B and mean was replaced with the method considered as the true value (FSP-Neo). The formula was used on all 25 measurements (19+6) and the mean of these results was defined as the correction factor. The corrected values of Bl_{orig} (**Bl_{corr}**) was calculated with the following formula:

$$Bl_{corr.} = Bl_{orig} / (1+(\text{'correction factor'}))$$

and applied to a new dataset. Further statistical analysis were performed with both corrected and non-corrected measurements ('Bl_{corr}' and 'Bl_{orig}' respectively) in order to demonstrate the consequences for the test results, if handheld devices are used in practice without bias are noticed. The background of the calculated correction factor and corrected data set is seen in Appendix B.

Prevalence

Prevalence of SCK on overall and herd level were calculated in RStudio within each of the four tests. SCK was defined with the following cutoff values: T_{milk}: ≥100 µmol/L[34], U_{man}: ≥1.5 mmol/L, U_{cat}: ≥1.5 mmol/L[34], Bl_{orig} and Bl_{corr}: ≥ 1.2 mmol/L[6,91] as proposed by scientists. Prevalence is a measurement of how frequent a condition is in a population at a particular time point.[92] In this case both the overall and herd level prevalence is the accumulated test-day prevalence:

$$\text{Overall/herd level prevalence} = \sum (\text{n positive cases/n total observations} *100\%)_{\text{test day n}}$$

Analysis of agreement between cowside tests (Bland Altman, Kappa and Coverage probability)

A Bland Altman analysis was performed in RStudio using the *bland.altman.plot* code, for demonstration of agreement between the calculated herd level prevalence of T_{milk}, U_{man} and U_{cat}, respectively, versus (vs.) both non-corrected (Bl_{orig}) and corrected (Bl_{corr}) measurements. The calculated herd level prevalence of the respective tests are demonstrated in Appendix C.

Cohen's kappa (κ) is a measure of agreement between two qualitative variables. It can be calculated when both methods have been used on the same sample. Weighted kappa (κ (*w*)) accounts the order of categories when the variable is on an ordinal scale. Values of κ range in the interval [0,1] (0=no agreement to 1=perfect agreement).[93] κ (*w*) has been calculated for the agreement of U_{man} and U_{cat} in Microsoft Excel using formula suggested in Houe et. al (2004).[93] κ(*w*) >0.8 was considered as high agreement. The interpretation of κ is general. Therefore, it is predetermined whether the agreement of two methods is good or bad. This calculation does not take any acceptable difference of context into account. For this reason, coverage probability (CP) has also been calculated.

CP is another measure of agreement. It describes the proportion of subjects that fall within a preset acceptable paired absolute difference. The advantage of CP is that it is simple, intuitive and easy to calculate. The user of CP has to set an acceptable difference a priori which is one major difference from other measurements of agreements such as κ. This may cause frustrations for users when using CP.[94] However, it forces the user to carefully consider the context of the evaluated test; how much difference do you accept under the present conditions?, and is thus presented for demonstration purposes in comparison to κ. CP of U_{man} and U_{cat} was calculated. CP was calculated using an analysis on the following link: *https://agreement-by-lin.shinyapps.io/Agreement_C/*. The acceptable difference was 0.5 categorical units. Allowance for calculated CP was 0.9: ≥90% of the differences must be ≤0.5 categorical units. CP of ≥0.90 was considered as high agreement.

Analysis of tests ability to detect ketosis (Sensitivity and Specificity)

Se and Sp of T_{milk}, U_{man}, and U_{cat} were calculated with different cutoffs against both Bl_{orig} and Bl_{corr}. SCK on cow level was defined as ≥1.2 mmol BHBA/L[6,91] Necessary values for calculation of Se and Sp were found in RStudio. Se and Sp were calculated in Microsoft Excel with formula suggested in Houe et al. (2004).[95]

Se is the probability that a truly positive subject will be classified as positive, using the test.
Sp is the probability that a truly negative subject will be classified as negative, using the test.[95]

Herd-based ketosis testing

According to Oetzel (2007) herd-based ketosis testing requires a subsample of ≥12 fresh cows (5 to 50 DIM) and then evaluate the proportion of cows above the alarm level (10%).[52] 10 of the 14 herds had sufficient sample sizes for categorization of ketosis status in the current study. The proportions of positive cows (≥1.2 mmol/L) with Bl_{orig} and Bl_{corr} in the 10 herds were calculated in RStudio, and demonstrated in a scatter plot with 95% confidence interval (CI) performed in Microsoft Excel. The used formula of proportions was suggested in Houe et al (2004).[92] The amount of negative, borderline, and positive herds were counted using a ≥10% alarm level as proposed by Oetzel (2007)

3.3. Study 2. Blood BHBA Concentrations in Dairy Cows in the Periparturient Period

The aim of study 2 was to investigate alternative sampling strategies in herds with ketosis problems.

3.3.1. Herd and animals

The study took place from October 14 until November 18, 2019 and was located at one dairy farm in the Middle-Western Jutland, Denmark. The farm consists of 500 dairy cows with an average milk yield of 12,000 kg ECM/cow/year. The dry cows are kept in a loose housing system on straw two weeks before calving and are fed dry cow feed (mainly straw and corn). The fresh cows are kept in a loose housing system on straw the first 14 days after calving (highly individual). Afterwards, they are transferred to a loose housing system with split concrete floors and beds with mattresses.
10 cows were included in the study. Selection of the cows was based on practical circumstances for the author (time schedule in the study period). The breed of the cows included Danish Holsteins, Danish Reds and mixed breeds. The cows were in first to fifth parity before calving (no heifers).

3.3.2. Sampling

Blood BHBA concentration (mmol/L) was measured three times a week. The intention was to obtain measurements two weeks before calving and two weeks after calving. In exactly two weeks before expected calving, the first measurement was collected on each cow. The cows were not measured on the days around calving in order to decrease the amount of stress for the cow during this period. The blood BHBA concentration was measured using the BHB-check device.
Two devices were used in this period (D1 and D2), because D1 broke down (former described). There was no locking system in the dry cow stable. Therefore the dry cows were transferred to a smaller stable section and total mixed ration (TMR) for the dairy cows were used as bait for blood collection. The data collection were done in the same matter as Bl_{orig} in study 1. When all samples were collected, the blood was transferred to the chamber of the electrochemical strip (<30 minutes

from the first obtained measurement). The blood BHBA concentration (mmol/L) was registered on a result scheme and transferred to an excel file in the end of the study period.

3.3.3. Statistical analysis

Statistical analysis were performed using Microsoft Excel for Macintosh (version 16.16.17 (191208)). The calculated correction factor from study 1 was used to correct the measurements obtained by D1. Further statistical analysis was based only on the corrected measurements.
A line chart of the variation in blood BHBA concentrations from the 10 cows was performed. Proportions of SCK in the first, second, and third measurements of the respective weeks pp were calculated using a cutoff value of ≥1.2 mmol/L blood BHBA, based on all measurements put together in weeks. Therefore, the measurements pp were divided in three measurements per week (1st to 3rd measurement=week 1, 4th to 6th measurements=week 2, 7th to 9th measurements=week 3, 10th measurement=week 4). Proportion was calculated with the following formula:

$$p = n \text{ positive cows } (\geq 1.2 \text{ mmol/L BHBA}) / n \text{ total}_{\text{test-day}}$$ [92]

A line chart that describes the risk of missing a case of SCK according to day of measurement was carried out, using measurements from one of the 10 cows. A comparison of ≥0.7 mmol/L blood BHBA prepartum with SCK (≥1.2 mmol/L BHBA) pp was made in accordance with Tatone et al., (2015)'s suggestion of this cutoff prepartum, when using a handheld device.[36]
The relative risk (RR) of developing SCK pp, when a cow had a measurement of ≥0.7 mmol/L blood BHBA prepartum, was calculated using the formula suggested in Houe et al. (2004).[96] The significance level was $p<0.05$. RR is the risk for a disease (SCK) in the exposed group (≥0.7 mmol blood BHBA/L prepartum) relative to the risk in the unexposed group (<0.7 mmol blood BHBA/L).[96] Raw data and background of results in study 2 are demonstrated in Appendix D.

4. Results

4.1. Pilot study

The first week was spend on the development of practical sampling technics; place an urinary catheter, perform vaginal stimulated micturition and practicing blood sampling from the coccygeal vein. The catheterization occupied much of the time and was the most challenging sample technic to practice. At the second day in the study period, the success for catheterization were approx.15% (3/20 fresh cows). In the 10th and final day the success were 89% (8/9 fresh cows). Vaginal stimulated micturition had high success after one morning of practice. Blood sampling for the BHB-check device had similar results as the stimulated micturition. The second week was spend on developing and practicing a routine for collection of data for study 1. No useful observations were registered during these two weeks of practice, because the practicing occupied all the time.

4.2. Study 1

14 herds with a total of 500 fresh cows were included in the study. 12 cows were excluded; 7 cows due to a repeated measurement, 3 cows due to wrong id-registration and 2 cows for not being categorized as a fresh cow (>21 DIM). Summary statistics of herds with number of cows according to parity, number of observations in each diagnostic test, number of cows with missing observations in one or more parameters, and number of cows with all four (complete) observations are given in table 3.

Table 3. Summary statistics of the sample population within herds: number of cows, distribution in parity group, distribution of observations in each diagnostic test, missing observations and complete (all four) observations.

	Cows (n)	1st parity (n)	2nd parity (n)	≥3rd parity (n)	T_{milk} (n)	U_{man} (n)	U_{cat} (n)	Bl_{orig} (n)	Missing obs.[1] (n)	Complete data (n)[2]
Herd 1	43	12	6	25	43	40	24	43	21	22
Herd 2	42	11	13	18	42	40	31	42	13	29
Herd 3	32	10	10	12	32	30	23	32	10	22
Herd 4	105	27	37	41	105	100	95	105	14	91
Herd 5	8	1	1	6	8	8	7	8	1	7
Herd 6	3	0	1	2	3	3	2	3	1	2
Herd 7	44	9	12	23	44	41	37	44	10	34
Herd 8	8	4	3	1	8	8	8	7	1	7
Herd 9	29	7	10	12	27	26	18	29	15	14
Herd 10	44	24	13	7	44	42	28	44	16	28
Herd 11	81	32	17	32	81	80	69	81	13	68
Herd 12	23	3	3	17	23	19	20	23	6	17
Herd 13	16	6	1	9	16	16	14	16	2	14
Herd 14	10	2	1	7	10	9	7	10	3	7
Total	488	148	128	212	486	462	383	487	126	362

n= number, [1]Missing observations in one or more parameter, [2]Cows with all four observations

126 cows had missing observations in one or more parameters. Reasons for missing observations are demonstrated in figure 3.

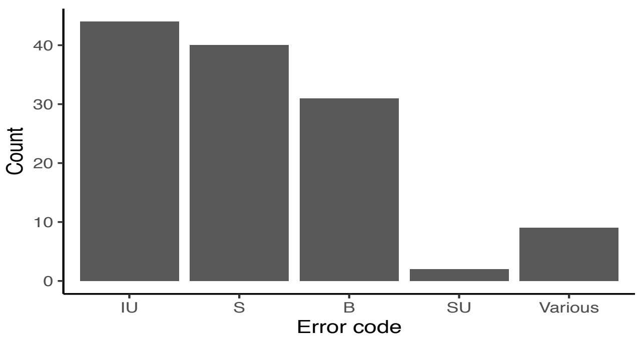


Figure 3. Histogram of error codes as reasons for missing observations in study 1.

Abbreviations: IU = 'no urine', S = kick of the cows, B = uterine infections (diagnosis and or putrid smell), SU = small urethra, Various = vaginal lacerations(2), diarrhea (1), rectal prolapse (1), IU + ketosis treatment (1), IU + B (1), S + bloody lochia discharge (1), IU+S (1) and unknown cause (1).

The three major reasons for missing observations according to diagnostic test is showed in figure 4.

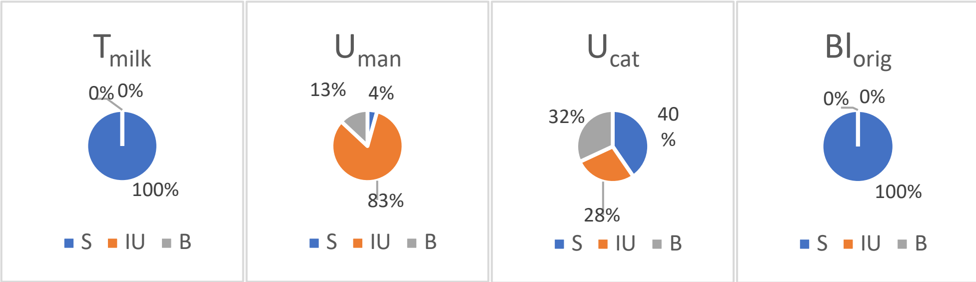


Figure 4. Distribution of the three major error codes according to diagnostic test.

Continuing statistic calculations are made from the complete dataset (362 fresh cows).

<u>Corrections factor</u>

The mean difference between photometrical evaluation and BHB-check were 0.193 mmol/L. The mean difference between photometrical evaluation and FSP-Neo were -0.04 mmol/L. Measurements of FSP-Neo were considered as 'gold'. The calculated correction factor was **0.857**. The correction factor was applied to a new dataset thereby correcting the measurements of Bl_{orig}. This was done via the formula mentioned in section 3.2.3. as follow:

$$Bl_{corr} = Bl_{orig} / (1+(0.857))$$

Only the D1 measurements were corrected (297 observations in the complete dataset). Further statistical analysis was performed with both corrected and non-corrected measurements in order to include the possible bias of the BHB-check device. It is crucial mentioning that the calculated mean correction factor of 0.857 is *only valid* in the current study, in order to perform further statistical evaluations against what may be considered as a 'gold standard'.

<u>Prevalence - overall level</u>

Table 4 shows the distribution of prevalence on overall level. Due to multiple decimals of Bl_{corr} compared to Bl_{orig}, the cutoff of blood BHBA concentration was set as ≥1.15 mmol/L (rounded= ≥1.2 mmol/L) in RStudio.

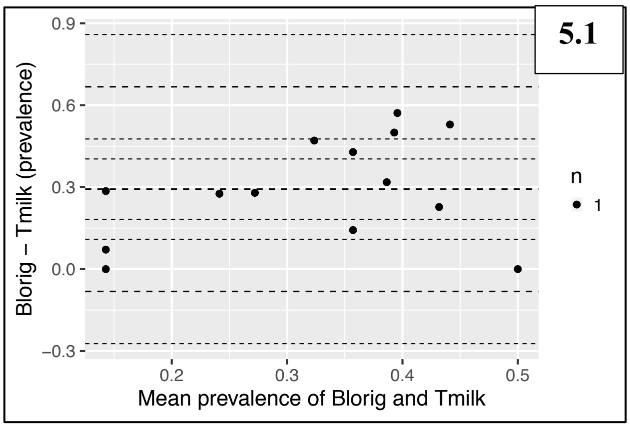
Table 4. Overall prevalence with T_{milk}, U_{man}, U_{cat}, Bl_{orig} and Bl_{corr} with 95% lower and upper confidence limits (LCL and UCL)

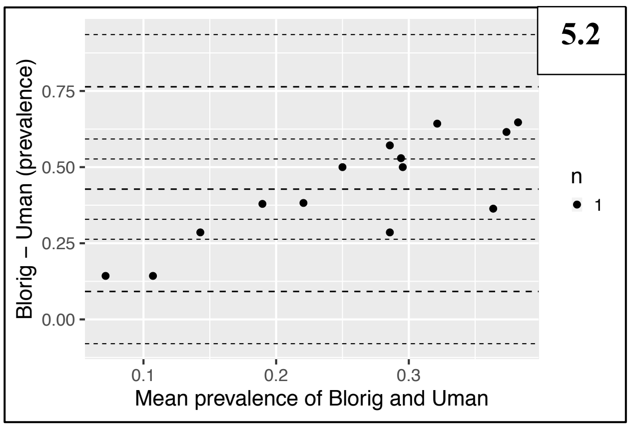
	Cutoff value	Prevalence	95% LCL	95% UCL
T_{milk}	≥100 µmol/L	0.138	0.103	0.174
U_{man}	≥1.5 mmol/L	0.047	0.025	0.069
U_{cat}	≥1.5 mmol/L	0.044	0.023	0.065
Bl_{orig}	≥1.2 mmol/L	0.506	0.454	0.557
Bl_{corr}	≥1.2 mmol/L	0.099	0.069	0.130

Prevalence of SCK on herd level

The agreement between the BHB-check and the three respective tests on herd level prevalence were examined. Bland Altman analysis of herd level prevalence of Bl_{orig} vs. T_{milk}, U_{man}, and U_{cat}, respectively are demonstrated in figure 5. Bland Altman analysis of herd level prevalence of Bl_{corr} vs. T_{milk}, U_{man}, and U_{cat}, respectively are demonstrated in figure 6. The difference between two tests is an estimate of the bias of one test relative to the other test.[90] If no differences were obtained between the mean herd prevalence of the respective tests, the plots would be distributed around 0. The limits of agreement were calculated as 95% confidence limits as proposed by Giavarina (2015). If the differences in the respective analysis are normally distributed, 95% of the differences will lie between these limits. Kolmogorov-Smirnov test of normality were used in RStudio to see if the differences were normally distributed (p-value <0.05 rejects normality).[90]

When analyzing the agreement of Bl_{orig} vs. T_{milk}, U_{man}, and Ucat, respectively, the estimated biases are positive (figure 5.1, 5.2, and 5.3, respectively). Therefore, the BHB-check estimates the prevalence 30%, 43%, and 43% higher than T_{milk}, U_{man}, and U_{cat}, respectively. The bias in all three tests is significant, since the line of equality (=0) is not in the 95% CI of the mean. None of the three analysis are normally distributed ($p<0.05$).





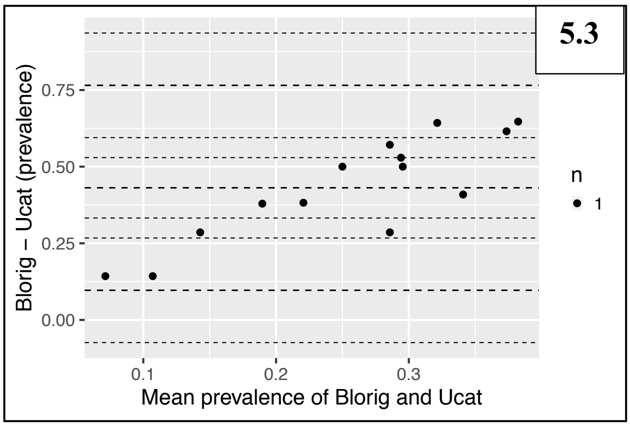


Figure 5. Herd-level prevalence (non-corrected)
Bland Altman plot with mean, 95% confidence interval limits for the mean and agreement limits.

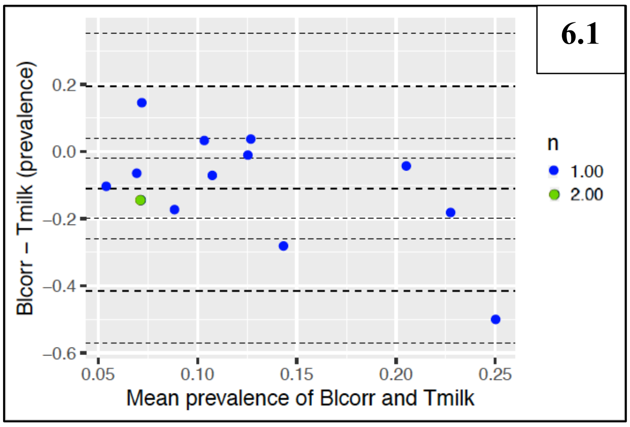
n: number of herds in the plot

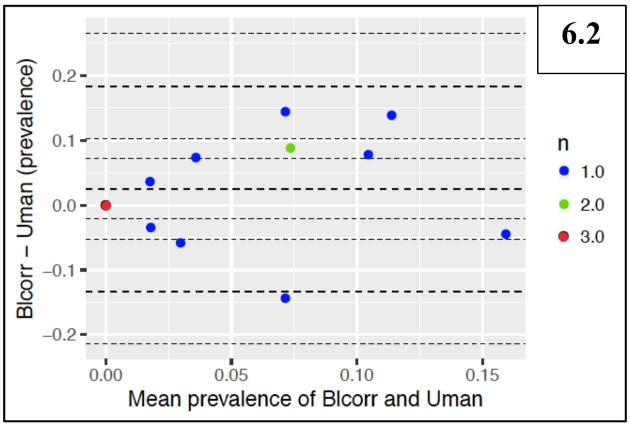
5.1: Bl_{orig} vs. T_{milk}: mean: +0.3, p=0.001581

5.2: Bl_{orig} vs. U_{man}: mean: +0.43, p=0.00007

5.3: Bl_{orig} vs. U_{cat}: mean: +0.43, p=0.00001

When analyzing the agreement of Bl_{corr} vs. T_{milk} the estimated bias is negative (-0.1) (figure 6.1). This means that KetoTest estimates the prevalence 10% higher than the BHB-check device. The mean difference of Bl_{corr} vs. U_{man} and U_{cat}, respectively are +0.02 in both analysis (figure 6.2 and 6.3, respectively). The bias of Bl_{corr} vs. T_{milk} is significant, since the line of equality is not in the 95% CI of the mean. The bias of Bl_{corr} vs. U_{man} and U_{cat}, respectively are not significant, since the line of equality is in the 95% CI of the mean in both analysis. Kolmogorov-Smirnov test of normality showed that all three analysis are normally distributed ($p>0.05$).





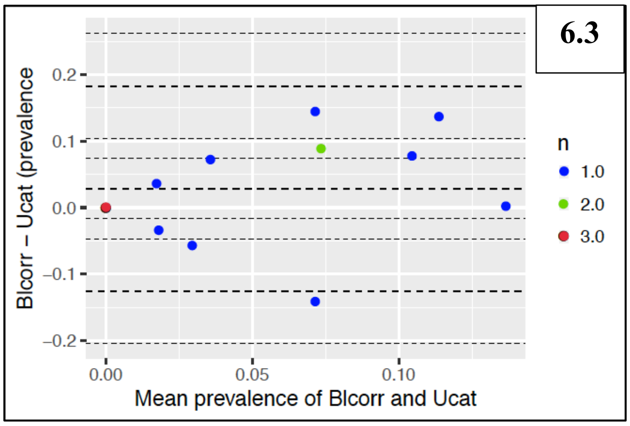


Figure 6. Herd-level prevalence (corrected)

Bland Altman plot with mean, 95% confidence interval for the mean and agreement limits.

n: number of herds in the plot

6.1: Bl_{corr} vs. T_{milk}: mean: -0.1, $p = 0.06039$

6.2: Bl_{corr} vs. U_{man}: mean: +0.02, $p = 0.3338$

6.3: Bl_{corr} vs. U_{cat}: mean: +0.02, $p = 0.3338$

<u>Weighted kappa and Coverage probability</u>

A κ (*w*) of 0.863 for U_{man} vs. U_{cat} was calculated and is demonstrated in table 5 with 95% lower and upper confidence interval (LCI and UCI, respectively). The calculated κ (*w*) indicates a high agreement between the two tests (κ (*w*)>0.8).[93]

Table 5. Weighted kappa κ (w) with, standard error (SE), lower (LCI) and upper confidence interval (UCI).

	κ (*w*)	SE	95% LCI	95% UCI
U_{man} vs. U_{cat}	0.863	0.174	0.553	~1

A CP of 0.952 was calculated for U_{man} vs. U_{cat}. Table 6 show the CP, 95% LCI and allowance. The calculated CP demonstrates high agreement between the two methods for urine collection (≥0.90).

Table 6. Coverage probability (CP) with lower confidence interval (LCI) and 90% allowance.

	$CP_{0.5}$	95% LCI	Allowance
U_{man} vs. U_{cat}	0.952	0.936	0.900

Sensitivity and specificity

Se and Sp of T_{milk}, U_{man}, and U_{cat} are calculated against the test considered as gold (Bl_{orig} and Bl_{corr}: ≥1.2 mmol/L blood BHBA/L) with different cutoff values. Results are shown in table 7 and table 8. Due to multiple decimals of Bl_{corr} compared to Bl_{orig}, the cutoff of BHBA concentration in Bl_{corr} was set as ≥1.15 mmol (rounded= ≥1.2 mmol/L) in RStudio.

Table 7. Sensitivity and specificity with 95% CI at different cutoff values in T_{milk}, U_{man}, and U_{cat} against Bl_{orig}.

Bl_{orig}	**Cutoff value**	**Se**	**95% CI**	**Sp**	**95% CI**
T_{milk}	≥50 µmol/L	0.22	[0.16;0.28]	0.87	[0.82;0.92]
	≥100 µmol/L	0.18	[0.13;0.24]	0.91	[0.86;0.95]
	≥200 µmol/L	0.02	[0.00;0.04]	0.97	[0.94;0.99]
U_{man}	≥0.5 mmol/L	0.37	[0.30;0.44]	0.92	[0.88;0.96]
	≥1.5 mmol/L	0.09	[0.05;0.14]	1.00	[1.00;1.00]
	≥4 mmol/L	0.04	[0.01;0.07]	1.00	[1.00;1.00]
U_{cat}	≥0.5 mmol/L	0.34	[0.28;0.41]	0.94	[0.90;0.97]
	≥1.5 mmol/L	0.09	[0.05;0.13]	1.00	[1.00;1.00]
	≥4 mmol/L	0.03	[0.01;0.06]	1.00	[1.00;1.00]

Table 8. Sensitivity and specificity with 95% CI at different cutoff values with T_{milk}, U_{man}, and U_{cat} against Bl_{corr}.

Bl_{corr}	**Cutoff value**	**Se**	**95% CI**	**Sp**	**95% CI**
T_{milk}	≥50 µmol/L	0.42	[0.27;0.59]	0.85	[0.81;0.89]
	≥100 µmol/L	0.33	[0.18;0.49]	0.88	[0.85;0.92]
	≥200 µmol/L	0	[0.00;0.00]	0.97	[0.95;0.99]
U_{man}	≥0.5 mmol/L	0.58	[0.42;0.74]	0.82	[0.77;0.86]
	≥1.5 mmol/L	0.25	[0.11;0.39]	0.98	[0.96;0.99]
	≥4 mmol/L	0.11	[0.01;0.21]	0.99	[0.98;1.00]
U_{cat}	≥0.5 mmol/L	0.58	[0.42;0.74]	0.84	[0.80;0.88]
	≥1.5 mmol/L	0.25	[0.11;0.39]	0.98	[0.96;0.99]
	≥4 mmol/L	0.08	[-0.01;0.17]	0.99	[0.98;1.00]

Herd-level testing

The proportions of positive cows (≥1.2 mmol/L BHBA) using Bl_{orig} and Bl_{corr} in the 10 herds are demonstrated in figure 7 and figure 8, respectively. The figures demonstrate scatter plots of the proportions with 95% CI, in the respective herds. The total amounts of herds according to the 10% alarm level for Bl_{orig} are 0/10 negative, 2/10 borderline and 8/10 positive herds. The total amounts of herds for $Bl_{corr.}$ are 3/10 negative, 7/10 borderline and 0/10 positive herds.

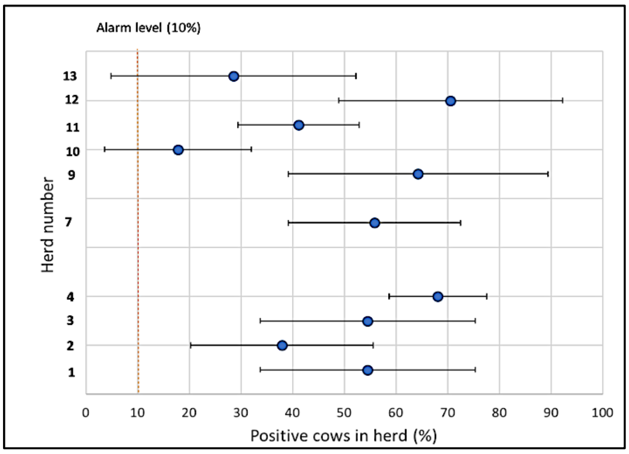


Figure 7. Scatter plots with 95% confidence interval demonstrating the proportion of positive cows (≥1.2 mmol/L blood BHBA) in the 10 herds with ≥12 animals. Alarm level: 10%. Non-corrected measurements.

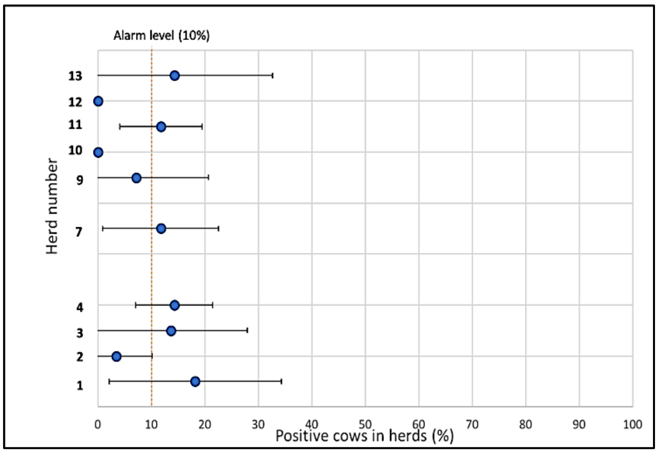


Figure 8. Scatter plots with 95% confidence interval demonstrating the proportion of positive cows (≥1.2 mmol/L blood BHBA) in the 10 herds with ≥12 animals. Alarm level: 10%. Corrected measurements.

4.3. Study 2

10 cows were included in the study. 12 measurements from each cow were obtained. Five cows had all measurements taken and five cows had missing measurements in one or several days. One cow (cow 2) was diagnosed with milk fever after calving. The treatment of the cow has not been registered. It was a downer cow the following two measurements and was kept in an isolated pen throughout the study period. Of the ten subjects, one cow calved as scheduled (cow_4), while the rest calved from up to 10 days before to 8 days later than expected.

The 12 measurements were obtained according to the schedule in spite of changes in the date of calving. Statistics are only performed with corrected measurements of D1. Figure 9 illustrates the variation of the blood BHBA concentration in the 10 cows during the study period.

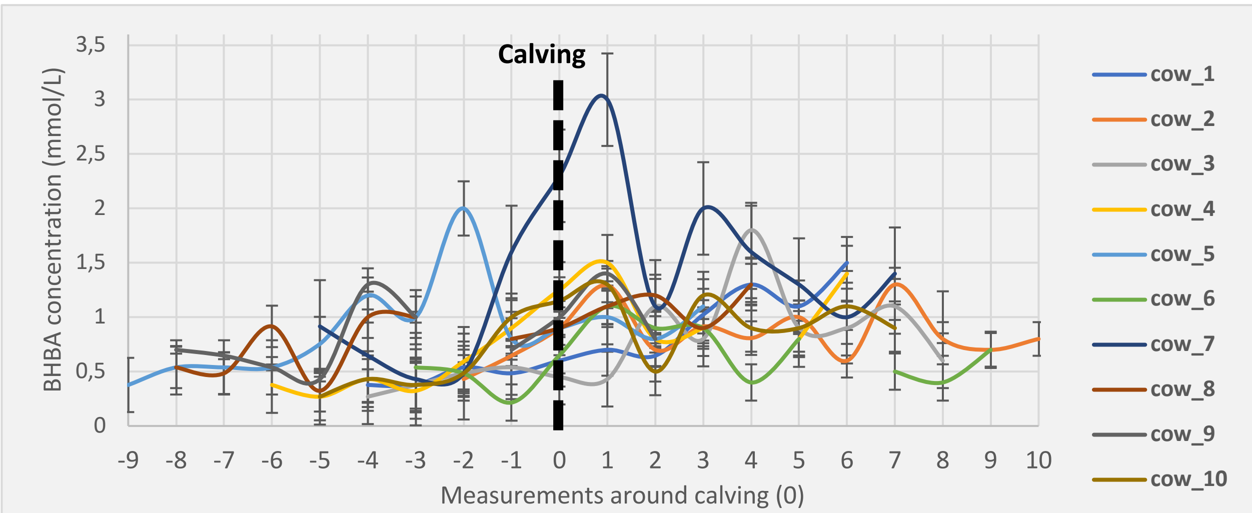


Figure 9. BHBA variation in blood obtained in 10 cows around calving (0). Measurements were obtained three times a week; two weeks prepartum and two weeks postpartum with 95% confidence interval.

The measurement interval –1 to 1 was between 3 and 4 days. The measurement interval 0 to 1 was between 1 and 2 days. Line chart with each cow separately can be seen in Appendix D-3.

Proportions of SCK (≥1.2 mmol blood BHBA/L) in weeks pp are seen in table 9. The proportions are calculated from all weekly measurements combined to four weeks pp.

Table 9. Proportion of cows with subclinical ketosis in week 1 to week 4 postpartum with three measurements a week (only one measurement in week 4)

Week postpartum	1			2			3			4
Measurement	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st
n	10	10	9	9	7	5	6	3	2	1
≥1,2 mmol/L	5	1	2	5	2	1	2	0	0	0
Proportion	0,50	0,10	0,22	0,56	0,29	0,20	0,33	0	0	0

Figure 10 demonstrates the risk of missing a case of SCK according to day of measurement: The cow develop SCK two times after calving (blue and green line). Within the first 5 to 6 DIM (red line) the cow develop SCK and returns to normal. If the cow is screened for ketosis on 5 to 6 DIM (third measurement pp) it would be diagnosed as healthy. If the screening is done once between 4 to 10 DIM, the second development of SCK (11 to 12 DIM) would not be diagnosed (green line).

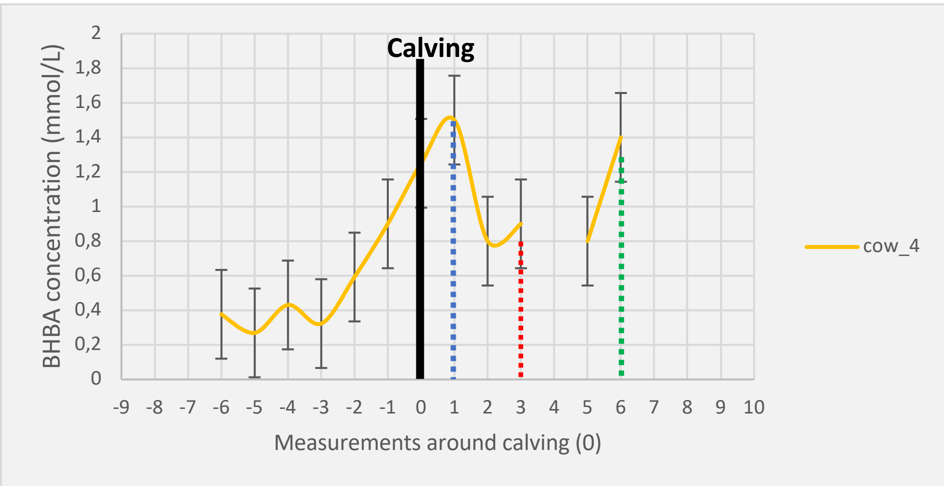


Figure 10. Blood BHBA variation (mmol/L) from cow 4 with 95% confidence interval. Blue line = Subclinical ketosis, the first time. Red line = Returned to normal. Green line = Subclinical ketosis, the second time.

The RR of developing SCK pp if a cow had one measurement of ≥0.7 mmol/L blood BHBA prepartum was 1.11 [0.569;2.170 95% CI]. This suggest a 1.11 higher risk of developing SCK pp, if measurements of ≥0.7 mmol/L blood BHBA are obtained one to three weeks prepartum. The findings are not significant (p=0.628).

5. Discussion

This thesis shows the importance of questioning the methods used in a practical setting. One cannot know for sure what caused the skewed measurement of D1 (the device itself? the batch of strips? how the blood samples were handled?). The BHB-check device was considered as gold based on the validation of the device.[68] In the technical note, it is only the BHB-check, and not the BHB-check plus device, which has been validated.[68] The devices used in this study were both BHB-check plus. Whether or not the validation counts for both editions is not certain to the author's knowledge. The ideal study include collection of blood samples from each fresh cow and subsequently handle and analyze them according to the recommendations.[97] In this way a 'true' gold standard would be found, which the cowside tests could be compared to. For financial limitations, this was not possible in the current study. Therefore, only six blood samples were photometrically analyzed, but along with the 19 field measurements, which all measured higher than the FSP-Neo device, there was a suspicion of a systemic bias in the BHB-check device used in the current study.
In order to trust the certainty of the discovered bias, the amount of blood samples send to the external laboratory should have been higher. If a reasonable guess of standard deviation is 0.26 mmol BHBA/L, a 95% CI is required ($Z_{1-\alpha/2}$=1.96), and the maximal allowable error is 0.1 mmol BHBA/L, then the appropriate sample size is: $(1.96^2 \times 0.26^2)/0.1^2 = \sim 26$ blood samples.[98]
Due to a possible systematic bias of the handheld device, and that the values considered as gold in the current study are calculated from a correction factor, the results must be read with precaution.

5.1. Pilot study

One of the purposes of the practical development in the study was to decrease the amount of time for examination of the cows and, in that way, be able to include more cows in study 1. Results of the pilot study showed that incorporation of time for practicing is beneficial. The catheterization of the cows turned out to be very difficult to learn and a large proportion of the practicing was spend on this diagnostic method. If the present study had been excluded, the number of fresh cows in study 1 would have been highly decreased.
The packets of KetoTest and Ketostix were kept opened in the belt when visiting the herds in the pilot study and in the first 2.5 days in study 1. The focus was kept on how to make the examinations as time efficient as possible. Even though the moisture bags remained in the packets of the test strips, there still might be a risk of contamination. If this had continued, it would decrease the reliability of the test results. The strips of U_{man} and U_{cat} in the pilot study, were read simultaneously

after the catheterization of the cows. This yielded a large time interval between reading of the test strips in U_{man} compared to U_{cat}. A delayed reading of the test strip may cause increased color intensity, which lower the Sp.[34,72] The reading of U_{man} was reduced to after approx. 15 s in study 1.

5.2. Study 1

Time of reading the semiquantitative test strips is an important factor to discuss. KetoTest and Ketostix must be read 60 s and 15 s after sample collection, respectively.[71,73] The time was counted manually. This is comparable with a practical setting, but it lowers the credibility due to a risk, that the Sp lowers with increased time.[72] The time interval for interpretation of T_{milk} and U_{man}, respectively, were highest in the beginning of the study period. As the author's efficiency increased in the study period, the time interval narrowed and the time for interpretation of the results of T_{milk} and U_{man}, respectively, were more uniform. Reason for delayed interpretation of T_{milk} and U_{man}, respectively, were due to complications in the catheterization of the cow.

<u>Missing observations</u>

Results of the reasons for missing observations showed that 'no urine' ('IU') accounted for 44 of the missing observations, followed by kicking of the cows and uterine infections (40 and 30, respectively). 'No urine' could either be due to incapability of the author to collect the urine, that the amount of urine in the cow was low, or the fact that the cattle practitioner also performed vaginal stimulated micturition, and therefore decreased the possibility for the author to collect the urine afterwards. 'No urine' accounted for 28% of the three major reasons for missing observations in U_{cat}. This is most likely due to lack of skills of the author, although it can be due to a low amount of urine in the cow in a few cases. A maximum of three attempt for catheterization were done. 40% of the three major reasons for missing observations in U_{cat} were due to kicking of the cow, which several times resulted in no more than one attempt. The kicks are probably due to a discomfort of the cow, which raises an ethical question about the catheterization. Primiparous cows accounted for approx. 1/3 of the total sample size (148 out of 488 cows). These animals might be more ill-tempered than multiparous cows, since the older cows are more used to human contact. This may increase the amount of missing observations due to kicking of the cows.

Uterine infections accounted for 13% in U_{man} and 32% in U_{cat} of the three major reasons for missing observations. If the cow was diagnosed with a uterine infection by the cattle practitioner or if the author discovered putrid smell of the vaginal discharge, no catheterization was performed. This threshold was adopted to decrease the risk of iatrogenic cystitis. In most cases of uterine infections, stimulated micturition could be performed, unless the cattle practitioner said otherwise or if the amount of discharge was too pronounced.

'Various' all resulted in missing observations of U_{man} and U_{cat}. If the cow had vaginal lacerations and or bloody mucoid discharge, the interpretation of U_{man} was difficult due to contamination of the test strip. If no precaution is made when applying the sample on the strip, a misinterpretation could easily occur. In these situations another diagnostic test method e.g. catheterization for urine collection, or ketone body measurement in milk or blood might be preferred.

Correction factor

The blood samples were stored at different temperatures after collection before the final diagnostic approach in the external laboratory was conducted. A study demonstrated that changes in BHBA concentration in heparinized whole blood under different temperatures (4°C and 24°C) were not significant.[99] 3 out of 6 blood samples had a hemolysis index (+ to ++++). A study showed that BHBA concentration were unaffected by any degree of hemolysis.[100] Both arguments increase the reliability of the measured BHBA concentrations performed by the external lab in the current study. FSP-Neo is developed for human use, but two studies have argued that it is useful in bovine blood. One study showed a Se of 100% and a Sp of 95% of FSP-Neo in the diagnosis of SCK in dairy cows (≥1.1 mmol/L serum BHBA).[87] Another study found strong agreement between FSP-Neo and standard laboratory methods.[86] Both facts are comparable with findings in the current study.

The calculated correction factor is based on the *mean* difference of proportion in which the BHB-check differs from the FSP-Neo. Therefore, using the correction factor is under the assumption that the difference between the devices are not dependent on the concentration level of BHBA in the blood. This means, that the correction factor does not take into account, whether or not the bias increases in variability according to blood BHBA concentrations.

The correction of Bl_{orig} was a necessary step in order to include the possible bias of the BHB-check device. Only the measurements of D1 were corrected. This was due to the fact, that this device was tested against measurements performed by the external lab. The agreement between measurements of D2 vs. T_{milk}, U_{man}, and U_{cat}, respectively, where more comparable than the measurements of D1 with the three tests, respectively. The accuracy of the D2 device is not investigated. Therefore, it is not certain that these measurements are reliable. Furthermore, when the shift to the D2 device occurred, a shift in batch of the electrochemical strips did also occurred. Therefore, it is not certain whether the calculated bias is caused by a random and or systematic error of the device, or due to a problem with the specific batch (or both). PortaCheck® was contacted to ask if they had experience problems with the specific batch number. No complaints were registered on the specific batch.

<u>Overall prevalence</u>

The overall prevalence of SCK was 50.6% (Bl_{orig}) and 9.9% (Bl_{corr}). The sample size was obtained in 14 herds with a total of 5,533 dairy cows in a part of one region in Denmark. The Danish population is approx. 569,000 dairy cows spread in all regions of the country.[101] Therefore, the calculated prevalence of SCK may not be representative for the entire Danish dairy cow population. Comparison of prevalence among studies may be difficult, because researchers have employed different ketosis thresholds, diagnostic methods, and sampling technics.[5] The overall prevalence of 50.6% is supported by one study from the Netherlands who found a ketosis prevalence of 58.8% using a serum BHBA of ≥1.2 mmol/L.[13] Another study found an overall SCK prevalence of 21.8% among 10 European countries, with a maximum and minimum prevalence of 36.6% and 11.2%, respectively.[16] The findings in the study differs from the SCK prevalence of Bl_{orig} in the current study(50.6%). They are more comparable with the SCK prevalence found by Bl_{corr} (9.9%). The overall prevalence of Bl_{corr} is further supported by the prevalence of 10% to 12% found by Krogh et al., (2011), though their prevalence was based on semiquantitative cowside tests on milk and urine, which may cause a lower true prevalence, due to decreased Se of these tests.[34,102] The SCK prevalence of Bl_{corr} is low compared to several other recent studies (20%[103] 21.8%[16] 58.8%[13]) based on ≥1.2 mmol/L blood BHBA. A reason for a relatively low prevalence of SCK of Bl_{corr} might be due to the fact that approx. 1/3 of the fresh cows in study 1 were primiparous cows. Suthar et al., (2013) showed that the odds of HK (≥1.2 mmol/L BHBA) was 1.2, 2.0 and 2.3 greater in parity 2, 3, and ≥4, respectively, than parity 1.

The overall prevalence of SCK performed by T_{milk} was 13.8%, which is higher than the prevalence of Bl_{corr} (9.9%). This finding is unexpected, due to the lower reported Se and Sp of KetoTest than urine and blood[34,68]. The overall prevalence of T_{milk} might be falsely increased. A reason may be due to SCC in the milk. In T_{milk}, the milk was sampled without prior testing of SCC in the milk. Increasing SCC may results in a false positive reaction of the test strip.[102] SCC testing in milk is often performed using a California Mastitis Test (CMT).[104] CMT was not done by the author prior to T_{milk} in study 1, but was often performed by the following cattle practitioner. If the data from the cattle practitioner were coupled with data of the current study (multivariate statistics) an association of T_{milk} results and SCC may be found. This requires though, that the chosen quarter of the udder had been mentioned in the data collection.

Another reason could be due to the storage of the KetoTest in study 1. The test strip should be stored at 2°C to 8°C and kept at room temperature before use.[73] Room temperature is highly variable in a stable and therefore temperature may influence the test strip. A study showed, that KetoTest had a κ (*w*) coefficient of 0.71 (0.62-0.80 95% CI) between both strip + milk at room temperature (24.0°C ±0.1°C)(gold) and cold strips (10.8°C ±0.9°C) + milk at room temperature. Further they showed, that the Se and Sp under these conditions were 0.74 and 0.75, respectively, when using blood BHBA concentration of ≥1.4 mmol/L.[105] This indicates a 25% risk of false positive when using cold KetoTest in 24.0°C milk.
Troubles with the interpretation of KetoTest were experienced, when the results were between score 10 to 12. Sometimes the color reaction of the test strip were not identical with the colors on the scale. The same decision of interpretation was repeated each time this occurred.

The overall prevalence of SCK by U_{man} and U_{cat}, respectively, were highly identical though the prevalence of U_{man} was slightly higher than U_{cat} (4.7% and 4.4%, respectively). This may either be explained by an interfering with lochial discharge on the test strip, which may cause a false interpretation of the color reaction, or an increased time interval for read of U_{man} as mentioned.[34]

Herd level prevalence

The agreements between prevalence on herd level of Bl_{orig} vs. T_{milk}, U_{man}, and U_{cat}, respectively, showed that Bl_{orig} estimates the prevalence higher than all three test (T_{milk}: 30%, U_{man}: 43%, and U_{cat}: 43%). Due to the higher Se and Sp of the BHB-check device than both KetoTest and Ketostix[34,68] a slightly higher prevalence of the BHB-check might be expected, but it is unlikely that it explains the differences obtained in the current study. Therefore, this overestimation supports the suspicion about a bias in the BHB-check (D1) on herd level.
Study 1 only includes 14 herds, but the plots are still supposed to be distributed in a smooth matter around the mean. This is not the case in any of the three analysis with Bl_{orig}. Analysis of agreements between Bl_{orig} vs. U_{man} and U_{cat}, respectively, show a lower difference between the two test methods when the mean prevalence is low. This suggests that the bias increases with higher measurements obtained by Bl_{orig}. None of the three analysis were normally distributed. Therefore, further caution in the interpretation of the differences between the respective tests should be taken.
The agreements between prevalence on herd level of Bl_{corr} vs. T_{milk}, U_{man}, and U_{cat}, respectively, yield a higher agreement between the BHB-check vs. all three tests compared to the results of Bl_{orig}. The bias of Bl_{corr} vs. U_{man} and U_{cat}, respectively, were not significant which suggest no differences

between the tests. T_{milk} estimated the prevalence 10% higher than the BHB-check, which may be explained by causes, that can influence the KetoTest as former described. The three analysis were normally distributed; 95% of the differences will be between the agreement limits (±1.96s).[90]

Weighted kappa and Coverage probability

κ(*w*) and CP were calculated to demonstrate agreement in two different statistical ways, to see whether they yield comparable results. Interpretation of the calculated κ(*w*) showed an agreement of 0.863. The calculated CP showed an agreement of 95.2%. These findings suggest that both statistical analysis yield a high agreement in spite of two different statistical approaches. The CP takes an acceptable difference (0.5 categorical units) into account compared to κ(*w*) where the interpretation is predetermined, based on a general assumption of what may be a high or low agreement. These high agreements between Ketostix on stimulated micturition and urinary catheterization show, that both collection methods may be used in a practical setting.

Sensitivity and specificity

The highest combined Se and Sp of KetoTest (T_{milk}) was obtained when using a cutoff value of ≥50 µmol/L in both Bl_{orig} (Se:0.22, Sp:0.87) and Bl_{corr} (Se:0.42, Sp:0.85). Both Se and Sp are lower than the Se and Sp of KetoTest reported by Carrier et al., (2004) (≥50 µmol/L Se:0.88, Sp:0.90).
The highest combined Se and Sp in the study were obtained using Ketostix with a cutoff of ≥0.5 mmol/L (U_{man}: Se:0.37, Sp:0.92 (Bl_{orig}) and Se:0.58, Sp:0.82 (Bl_{corr}); U_{cat}: Se:0.34, Sp:0.94 (Bl_{orig}) and Se:0.58, Sp:0.84 (Bl_{corr})). All Se are lower than the Se of Ketostix reported by Carrier et al., (2004) (≥0.5 mmol/L, Se:0.90, Sp:0.86) and Iwersen et al., (2009) (≥0.5 mmol/L, Se:0.78, Sp:0.92). Increasing the cutoff value in all three tests in both Bl_{orig} and Bl_{corr} will increase the Sp but lower the Se. Both Carrier et al., (2004) and Iwersen et al., (2009) used a SCK threshold in serum of ≥1.4 mmol BHBA/L.[34,89] One study showed that the Se of Ketostix decreased from 0.72 to 0.44 when changing the SCK threshold from 1.4 mmol to 1.1 mmol serum BHBA/L.[106] If Carrier et al. (2004) and Iwersen et al. (2009) lowered the SCK threshold of blood BHBA or the SCK threshold was increased to ≥1.4 mmol blood BHBA/L in the current study, more similar results might be found.

In a practical view, the cutoffs that yielded the highest Se and Sp for both KetoTest and Ketostix (≥50µmol/L and ≥0.5 mmol/L, respectively), might not be transferable to a practical setup. Both cutoffs equal the second score on the color chart, which only slightly differ in color from a negative score. In a practical setting, where diagnostics are done relatively fast, a distinguish between 'negative' and the first color reaction might not be easy to obtain.

The Se of KetoTest (≥50 µmol/L) were lower than the Se of Ketostix (≥0.5 mmol/L) when using Bl_{corr}. Diurnal variation of BHBA concentration in milk might influence the test results. Nielsen et al., (2003) demonstrated that the milk BHBA concentration were highest at milking and lowest between milkings.[61] This is important, when analyzing Se and Sp of diagnostic tests based on multiple collected samples over time. If samples were collected at different time points during the day, in the different weeks of the study period, the diurnal variation would be a marked bias of the study. The herds were visited at the same time in the respective weeks, which lower the bias caused by diurnal variation. A correlation coefficient of 0.66 between blood BHBA and milk BHBA has been demonstrated.[62] This might also cause a lowered Se of ketosis screening in milk.
The Sp of KetoTest (≥50µmol/L) were higher than Sp of Ketostix (≥0.5mmol/L) when using Bl_{corr} (0.85 and 0.82, respectively). This is supported by Carrier et al., (2004), and suggest that KetoTest find less false positive cows than Ketostix. When increasing the cutoffs, the opposite is seen.

Se and Sp of T_{milk}, U_{man}, and U_{cat}, respectively, was calculated using Bl_{orig} and Bl_{corr} as gold standards. A major reason for the relatively low Se and Sp of T_{milk}, U_{man}, and U_{cat}, respectively, in study 1 is most likely due to lack of a true gold standard. The Se and Sp analysis could be performed again with a different approach, using latent class analysis. Latent class analysis is a statistical approach to estimate Se and Sp of diagnostic tests, without the assumption of one being a gold standard.[7,107] This thesis did not included this type of analysis.

Herd-level testing

70% of the 10 included herds were borderline, when using Bl_{corr} and an alarm level of 10% in herd-level ketosis testing. If the herd is classified as borderline, additional sampling might be beneficial, although it is important to compare the test results with clinical appearance in the herd and weight the cost-benefits.[52] The results supports the fact that ketosis prevalence is herd-related, and that the prevalence vary between herds. This ketosis testing strategy is not designed to optimize a feeding program for prevention of ketosis, but is intended to identify herds with either very high or very low ketosis prevalence (figure 3 in Oetzel (2007)).[52] The cutoff value of positive cows was ≥1.2 mmol/L blood BHBA. Oetzel (2007) have used a cutoff value of ≥1.4 mmol/L blood BHBA, which one should keep in mind if using the figure for interpretation of result in the current study.

5.3. Study 2

The blood BHBA variation of the cows in the transition period, obtained in the current study, is an important aspect to keep in mind when performing ketosis screening in dairy herds. The obtained sample size is very small, so the findings may not be representative for all transitional dairy cows. Due to biological variation of due dates, it was impossible to follow the scheduled measurements. Therefore, in order to compare the findings from the cows, it was necessary to combine the measurements from -9days to +10 day even though, in reality, the last measurement of cow 10 was obtained 38 days later than the measurement of cow 1. This caution should be taken.

The blood was sampled in syringes from all the cows and read with the BHB-check device after collection, simultaneously. Therefore, the blood was kept in the syringes at temperature according to the weather and some of the blood were partly clotted when analyzed with the meter. One study showed that storage and temperature of clotted blood did not affect the BHBA concentration.[108] The proportions in the first week pp varies highly according to the day of measurement. The highest proportions were found in the first measurement after calving (first measurement in week 1) and in the fourth measurement after calving (first measurement in week 2) (0.50 and 0.56, respectively). These measurements were obtained at 1 to 2 DIM and 7 to 8 DIM, respectively. These findings are supported by the cumulative prevalence of SCK (≥1.2 mmol/L blood BHBA) found by McArt et al., (2012) (=3 to 10 DIM) in dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM.[6]

The variation in BHBA concentration in cow 4 (figure 10) is similar to reported variations found by other studies.[109,110] The cow develops SCK and returns to normal within 5 days. This supports the median length of a SCK case of 5 days found by McArt et al., (2012). In the included herd, the 'routine-examination-visits' were performed once at 4 to 10 DIM. In this specific case, if screening is done once at 4 DIM, the cow would be diagnosed as healthy, even though it develops SCK twice after calving. This is an example of incidence and prevalence. Incidence describes how quickly new cases develop in a specific time period while prevalence describes the proportions of tested animals that have SCK at the time of testing.[92] Incidence requires frequent testing, which increases the costs for screening. Therefore, prevalence is often preferred. The incidence of SCK is about 2.2 times the average prevalence of SCK and is a useful tool for decision of individual animal treatment.[3,6] One study showed that in herds with a SCK incidence of 15-50% (≥1.2 mmol/L blood BHBA) it is most financial to test cows twice a week from 3 to 9 DIM and treating SCK with 300mL oral propylene glycol for 5 d.[111] These recommendations could be followed in herds with high SCK occurrence.

RR of developing SCK pp, if a cow had a measurement of ≥0.7 mmol/L blood BHBA prepartum was 1.11. This suggests, that SCK prevention should include the dry period, but the finding was not significant. The sample size has to be increased in order to demonstrate this association.

The use of the handheld device

The bias of the device (or batch of electrochemical strips) and the breakdown of the device due to a blood contamination inside the meter are both highly inconvenient and disabling aspects for a diagnostic method in a practical setting. The discovery of the possible inaccuracy of the device was a result of the authors wondering about the relatively high measurements obtained by the device, combined with the following cattle practitioners knowledge to the herds and his years of work experience ('evidence-based work').[112] This demonstrates the importance of testing the accuracy of used diagnostic procedures consequently and regularly e.g. by collection of blood samples to laboratory analysis.

When using the BHB-check device it was difficult to ensure clean and dry hands, when applying a test strip in the meter as recommended by the manufacture.[67] Problems with error codes in the device were experienced (most often either a problem with the strip or 'temperature too low'). This increases the expenses due to waste of strips and time spend on heating the device. Price is an important aspect when comparing diagnostic tests. Testing one cow using the BHB-check costs approx. 14.5 DKK (+ the price of the device and the number of times it can be used before it breaks down) compared to 12 DKK (KetoTest) and 2 DKK (Ketostix). Thus, the advantages of handheld devices has to be relatively high. The advantage of using milk is, that it is easy to sample. Urine might be difficult to sample, and interfering with lochial discharge may give false positive results.

6. Conclusion

Data collection is challenging and should not be taken for granted. Urine sampling was difficult, particularly the urinary catheterization of a cow. Stimulated micturition caused less kicking of the cows, which could argue that this way of collection is more ethical correct. The handheld device was easy to use, but the strips require clean hands which was difficult to maintain among cows.

Semiquantitative tests used in urine underestimated the prevalence of SCK compared to quantitative tests. Semiquantitative tests used in milk overestimated the prevalence of SCK compared to the quantitative test (corrected measurements). When using semiquantitative tests on milk or urine, it is important to check for influential factors such as SCC and diurnal variation in milk, and uterine infections when using urine dipsticks. Overall, screening of ketosis on urine was more sensitive than ketosis screening on freshly sampled milk, but varied highly depending on cutoff values.

Ketosis screening on vaginal stimulated micturition and on catheterized urine showed high agreement, although not total agreement. Vaginal discharge may interfere the test results.

Variation in periparturient BHBA concentrations might result in missing cases of SCK. Cows in the current study developed SCK more than once in the pp period. If ketosis screening is only done once and in between these developments, the cow will not be noticed. Screening cows more than once may detect more positive cows, although the cost-benefit has to be weighted.

No significant association between elevated blood BHBA prepartum and SCK postpartum was found. A larger sample size is required to detect this association.

Due to the bias of the handheld device and the calculated 'gold' measurements, the findings in the current study should be read with high precaution. The bias of the handheld device demonstrates the importance of testing the accuracy of diagnostic procedures on a regularly basis.

7. Future perspectives

The calculated correction factor was used under the assumption that the bias was not dependent on the BHBA level in the blood. The six blood samples from the external laboratory yielded a suspicion, that it was not the fact (Appendix B-1). Furthermore, the 19 field measurements questioned the precision of the BHB-check device, due to the fact, that identical BHBA concentrations, measured with the FSP-Neo, measured differently with the BHB-check. Further work would include an investigation of the precision and accuracy of the BHB-check device with repeated measurements in both high, middle and low levels of blood BHBA concentrations.

The Se and Sp analysis could be performed again with a different approach, using latent class analysis as former described.

Disposable plastic catheters were used, which removes the need for disinfection. The length of these catheters often made a rectal examination necessary to make the urine flow (time consuming and unnecessary for this study). Alternatively, a metal urinary catheter for cows could have been used. The latter require high level of disinfection (very time consuming).

If the study was to be performed again, it would be recommended to involve at least two persons for the data collection, thereby increasing the diagnostic reliability (one person for collection and one for interpretation).

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9. Appendices

Appendix A – Raw data of study 1

A-1. Raw data set of study 1

Table A. Raw data of study 1. *NA = missing observations , Yellow row = shift to D2 device*

Herd-id	Cow-id	Parity	Test 1	Test 2	Test 3	Test 4	Error code	Date	Parity group
1	1	parity_4	12	2	2	1,5		16/09/2019	Older
1	2	parity_1	10	1	NA	0,9	S	16/09/2019	First
1	3	parity_1	10	1	1	1,1		16/09/2019	First
1	4	parity_5	10	2	NA	1	B	16/09/2019	Older
2	5	parity_2	12	1	1	1,1		16/09/2019	Second
2	6	parity_2	10	1	NA	1,1	S	16/09/2019	Second
2	7	parity_4	10	1	1	0,8		16/09/2019	Older
2	8	parity_1	10	1	1	0,8		16/09/2019	First
2	9	parity_1	10	1	1	0,9		16/09/2019	First
2	10	parity_3	10	1	1	1,3		16/09/2019	Older
3	11	parity_5	10	1	1	1,1		16/09/2019	Older
3	12	parity_3	12	4	4	2,2		16/09/2019	Older
3	13	parity_2	10	1	1	0,9		16/09/2019	Second
3	14	parity_2	10	1	1	1,3		16/09/2019	Second
3	15	parity_1	10	1	1	1		16/09/2019	First
4	16	parity_3	13	2	2	1,3		17/09/2019	Older
4	17	parity_1	12	4	4	1,6		17/09/2019	First
4	18	parity_1	10	1	NA	1,4	SU	17/09/2019	First
4	19	parity_3	10	2	2	1,8		17/09/2019	Older
4	20	parity_2	10	1	NA	1,3	SU	17/09/2019	Second
4	21	parity_2	10	1	1	1		17/09/2019	Second
4	22	parity_8	10	1	1	1,5		17/09/2019	Older
5	23	parity_3	10	1	1	1,7		17/09/2019	Older
5	24	parity_3	10	1	1	0,9		17/09/2019	Older
5	25	parity_2	10	1	1	1		17/09/2019	Second
5	26	parity_5	10	2	1	1,8		17/09/2019	Older
5	27	parity_4	10	1	1	1,8		17/09/2019	Older
6	28	parity_4	12	1	1	1,3		18/09/2019	Older
6	29	parity_2	10	2	NA	0,9	S	18/09/2019	Second
6	30	parity_4	10	2	1	0,9 parity_		18/09/2019	Older
7	31	parity_3	10	1	1	0,8		18/09/2019	Older
7	32	parity_2	10	NA	1	0,4	IU	18/09/2019	Second
7	33	parity_3	10	2	2	1,4		18/09/2019	Older

7	34	parity_5	10	NA	1	0,9	IU	18/09/2019	Older
7	35	parity_1	10	1	1	1,2		18/09/2019	First
7	36	parity_2	10	1	1	0,7		18/09/2019	Second
7	37	parity_3	10	1	1	0,8		18/09/2019	Older
7	38	parity_3	10	1	NA	1,2	B	18/09/2019	Older
8	39	parity_1	10	1	1	NA	S	18/09/2019	First
8	40	parity_1	10	1	1	0,8		18/09/2019	First
8	41	parity_1	10	1	1	0,5		18/09/2019	First
8	42	parity_2	12	1	2	1,5		18/09/2019	Second
8	43	parity_1	10	1	1	0,8		18/09/2019	First
8	44	parity_3	12	3	3	2,1		18/09/2019	Older
8	45	parity_2	10	1	1	1,6		18/09/2019	Second
8	46	parity_2	10	1	1	0,8		18/09/2019	Second
9	47	parity_3	10	1	1	1,6		19/09/2019	Older
9	48	parity_3	10	1	1	1,1		19/09/2019	Older
9	49	parity_2	10	1	1	1,4		19/09/2019	Second
9	50	parity_3	10	1	1	1,5		19/09/2019	Older
9	51	parity_2	10	2	2	1,3		19/09/2019	Second
9	52	parity_2	10	1	1	1,6		19/09/2019	Second
9	53	parity_4	10	1	NA	0,9	S	19/09/2019	Older
9	54	parity_2	12	1	1	1,2		19/09/2019	Second
2	55	parity_1	10	1	1	1,1		23/09/2019	First
2	56	parity_3	10	1	1	0,9		23/09/2019	Older
2	57	parity_2	10	1	1	1		23/09/2019	Second
2	58	parity_2	10	1	1	1,1		23/09/2019	Second
2	59	parity_6	11	1	NA	1,1	S	23/09/2019	Older
1	60	parity_1	13	1	1	1,1		23/09/2019	First
1	61	parity_4	10	1	NA	0,9	IU	23/09/2019	Older
1	62	parity_1	10	1	1	0,8		23/09/2019	First
1	63	parity_1	10	2	NA	1,1	S	23/09/2019	First
1	64	parity_2	10	1	1	1,2		23/09/2019	Second
1	65	parity_1	10	2	NA	1,6	B	23/09/2019	First
1	66	parity_2	10	1	1	1,2		23/09/2019	Second
1	67	parity_2	12	2	2	1,3		23/09/2019	Second
4	68	parity_4	10	NA	2	0,5	IU	24/09/2019	Older
4	69	parity_1	10	1	1	0,7		24/09/2019	First
4	70	parity_1	13	1	1	1,3		24/09/2019	First
4	71	parity_3	12	4	4	2,6		24/09/2019	Older
4	72	parity_1	10	1	1	1,3		24/09/2019	First

4	73	parity_3	10	2	2	1,6		24/09/2019	Older
4	74	parity_3	10	1	1	0,8		24/09/2019	Older
4	75	parity_1	10	1	1	1,1		24/09/2019	First
5	76	parity_4	10	1	NA	0,9	IU	24/09/2019	Older
5	77	parity_3	10	1	1	1,3		24/09/2019	Older
5	78	parity_1	12	2	1	1,1		24/09/2019	First
10	79	parity_1	10	1	1	0,8		25/09/2019	First
10	80	parity_3	12	1	1	1		25/09/2019	Older
10	81	parity_2	10	1	1	0,7		25/09/2019	Second
10	82	parity_1	10	1	1	1,2		25/09/2019	First
10	83	parity_2	10	1	NA	0,9	B	25/09/2019	Second
10	84	parity_4	10	1	1	0,7		25/09/2019	Older
10	85	parity_1	10	1	NA	0,4	UD	25/09/2019	First
10	86	parity_2	12	4	4	1,8		25/09/2019	Second
10	87	parity_1	10	1	NA	1	IU	25/09/2019	First
10	88	parity_2	10	1	1	1		25/09/2019	Second
10	89	parity_1	10	1	NA	0,6	B	25/09/2019	First
10	90	parity_1	10	1	1	0,8		25/09/2019	First
10	91	parity_1	10	NA	NA	0,7	B	25/09/2019	First
10	92	parity_2	12	NA	NA	1,7	UE	25/09/2019	Second
10	93	parity_1	10	1	1	0,4		25/09/2019	First
10	94	parity_1	10	1	1	0,9		25/09/2019	First
10	95	parity_2	10	1	1	1,4		25/09/2019	Second
10	96	parity_2	10	2	2	1,1		25/09/2019	Second
11	97	parity_3	10	1	1	1,2		26/09/2019	Older
11	98	parity_4	12	1	1	1,2		26/09/2019	Older
11	99	parity_3	12	5	5	4,2		26/09/2019	Older
11	100	parity_3	10	1	1	0,6		26/09/2019	Older
11	101	parity_2	10	2	1	0,7		26/09/2019	Second
11	102	parity_2	10	1	1	0,5		26/09/2019	Second
11	103	parity_1	10	1	1	0,6		26/09/2019	First
11	104	parity_1	10	1	1	1,1		26/09/2019	First
11	105	parity_1	10	1	1	0,5		26/09/2019	First
11	106	parity_1	10	1	1	0,5		26/09/2019	First
11	107	parity_1	10	1	1	0,9		26/09/2019	First
9	108	parity_3	10	1	NA	0,9	IU	26/09/2019	Older
9	109	parity_2	NA	2	NA	1,4	S	26/09/2019	Second
9	110	parity_5	14	1	1	1,5		26/09/2019	Older
9	111	parity_1	10	1	NA	0,7	S	26/09/2019	First

9	112	parity_5	10	1	NA	1,6	B	26/09/2019	Older
9	113	parity_3	10	NA	2	1,6	IU	26/09/2019	Older
9	114	parity_3	10	2	NA	1,7	IU	26/09/2019	Older
9	115	parity_2	10	1	2	1		26/09/2019	Second
2	116	parity_1	10	1	1	1		30/09/2019	First
2	117	parity_4	12	1	1	1		30/09/2019	Older
2	118	parity_1	10	1	1	1		30/09/2019	First
2	119	parity_4	10	1	1	1,4		30/09/2019	Older
2	120	parity_2	10	1	1	1,4		30/09/2019	Second
1	121	parity_6	10	2	NA	0,9	IU	30/09/2019	Older
1	122	parity_3	10	1	1	0,8		30/09/2019	Older
1	123	parity_3	10	NA	NA	0,9	IU	30/09/2019	Older
1	124	parity_3	10	1	1	0,7		30/09/2019	Older
1	125	parity_3	10	1	NA	0,5	S	30/09/2019	Older
3	126	parity_5	10	1	NA	1,8	IU	30/09/2019	Older
3	127	parity_4	12	2	2	1,9		30/09/2019	Older
3	128	parity_2	12	1	1	1,4		30/09/2019	Second
3	129	parity_2	12	1	1	1,1		30/09/2019	Second
3	130	parity_2	10	3	2	1,6		30/09/2019	Second
3	131	parity_2	10	1	1	1,4		30/09/2019	Second
3	132	parity_1	10	1	1	1,1		30/09/2019	First
4	133	parity_3	10	1	1	0,7		01/10/2019	Older
4	134	parity_4	12	2	1	2		01/10/2019	Older
4	135	parity_4	10	1	NA	1,4	S	01/10/2019	Older
4	136	parity_2	10	2	2	1,6		01/10/2019	Second
4	137	parity_2	10	1	1	1,4		01/10/2019	Second
4	138	parity_2	10	NA	3	1,3	IU	01/10/2019	Second
4	139	parity_2	10	2	2	2,2		01/10/2019	Second
4	140	parity_2	10	1	1	1,6		01/10/2019	Second
4	141	parity_2	10	1	1	1		01/10/2019	Second
4	142	parity_1	10	1	1	1,4		01/10/2019	First
4	143	parity_3	10	2	2	2,1		01/10/2019	Older
4	144	parity_1	10	1	1	1		01/10/2019	First
4	145	parity_1	10	1	1	1,3		01/10/2019	First
4	146	parity_1	10	1	1	0,9		01/10/2019	First
4	147	parity_1	10	1	1	1,1		01/10/2019	First
4	148	parity_1	10	1	1	1,8		01/10/2019	First
12	149	parity_3	12	NA	NA	1,4	S	01/10/2019	Older
12	150	parity_5	12	NA	3	3,1	IU	01/10/2019	Older

12	151	parity_3	10	1	1	1,3		01/10/2019	Older
12	152	parity_1	10	1	2	1,2		01/10/2019	First
12	153	parity_2	12	1	1	1,2		01/10/2019	Second
12	154	parity_4	13	1	1	1		01/10/2019	Older
12	155	parity_3	11	1	2	0,9		01/10/2019	Older
12	156	parity_5	10	NA	1	0,6	IU	01/10/2019	Older
12	157	parity_3	12	NA	2	1,3	IU	01/10/2019	Older
12	158	parity_4	10	1	1	1,6		01/10/2019	Older
12	159	parity_4	10	1	1	1,4		01/10/2019	Older
12	160	parity_4	10	1	1	1,6		01/10/2019	Older
7	161	parity_5	10	1	1	1,1		02/10/2019	Older
7	162	parity_5	10	1	1	1,6		02/10/2019	Older
7	163	parity_5	10	1	NA	0,9	IU	02/10/2019	Older
7	164	parity_5	10	1	1	1,7		02/10/2019	Older
7	165	parity_4	10	1	NA	0,9	B	02/10/2019	Older
7	166	parity_3	10	1	1	1		02/10/2019	Older
7	167	parity_2	10	1	1	1		02/10/2019	Second
7	168	parity_2	10	1	1	1,4		02/10/2019	Second
11	169	parity_1	12	2	1	0,8		03/10/2019	First
11	170	parity_3	10	1	1	0,7		03/10/2019	Older
11	171	parity_1	10	1	NA	1,4	UD	03/10/2019	First
11	172	parity_1	10	1	1	0,8		03/10/2019	First
11	173	parity_4	10	1	1	1,2		03/10/2019	Older
11	174	parity_3	10	1	1	1,3		03/10/2019	Older
11	175	parity_3	10	1	1	0,6		03/10/2019	Older
11	176	parity_3	12	4	3	2,3		03/10/2019	Older
11	177	parity_2	10	1	1	1		03/10/2019	Second
11	178	parity_2	14	5	NA	6	S	03/10/2019	Second
13	179	parity_4	10	1	1	1		04/10/2019	Older
13	180	parity_4	10	1	1	1,3		04/10/2019	Older
13	181	parity_4	10	1	1	1		04/10/2019	Older
13	182	parity_1	10	2	1	1,2		04/10/2019	First
13	183	parity_1	10	1	1	0,9		04/10/2019	First
1	184	parity_4	10	1	NA	1,4	S	07/10/2019	Older
1	185	parity_1	10	1	1	1,7		07/10/2019	First
1	186	parity_3	10	1	NA	1,6	S	07/10/2019	Older
2	187	parity_3	10	1	NA	1	S	07/10/2019	Older
2	188	parity_1	13	1	1	1		07/10/2019	First
2	189	parity_2	10	1	1	1,5		07/10/2019	Second

2	190	parity_5	10	1	1	1,4		07/10/2019	Older
4	191	parity_5	10	1	1	1,3		08/10/2019	Older
4	192	parity_4	11	2	2	1,1		08/10/2019	Older
4	193	parity_4	10	1	1	1,6		08/10/2019	Older
4	194	parity_4	11	2	2	2,3		08/10/2019	Older
4	195	parity_4	10	1	1	1,6		08/10/2019	Older
4	196	parity_3	10	2	2	1,5		08/10/2019	Older
4	197	parity_3	10	1	1	1,5		08/10/2019	Older
4	198	parity_2	10	1	1	1,1		08/10/2019	Second
4	199	parity_1	10	1	1	1,1		08/10/2019	First
4	200	parity_1	10	1	1	1,6		08/10/2019	First
4	201	parity_2	13	1	1	1,5		08/10/2019	Second
4	202	parity_2	10	1	1	0,5		08/10/2019	Second
4	203	parity_2	11	3	3	2,2		08/10/2019	Second
4	204	parity_2	10	1	1	1,3		08/10/2019	Second
4	205	parity_2	10	1	1	1,4		08/10/2019	Second
4	206	parity_2	10	1	1	1,4		08/10/2019	Second
4	207	parity_2	10	1	1	1,1		08/10/2019	Second
4	208	parity_2	10	1	1	1,4		08/10/2019	Second
4	209	parity_1	10	1	1	1,1		08/10/2019	First
4	210	parity_1	10	1	1	0,9		08/10/2019	First
10	211	parity_3	10	1	1	0,8		09/10/2019	Older
10	212	parity_2	10	1	1	1,1		09/10/2019	Second
10	213	parity_1	10	1	1	0,8		09/10/2019	First
10	214	parity_1	10	1	1	0,8		09/10/2019	First
10	215	parity_4	10	2	2	1,5		09/10/2019	Older
10	216	parity_1	10	1	1	1,2		09/10/2019	First
10	217	parity_2	10	1	NA	1,1	S	09/10/2019	Second
10	218	parity_1	10	1	NA	0,8	S	09/10/2019	First
10	219	parity_5	10	1	1	0,8		09/10/2019	Older
7	220	parity_4	10	1	2	1,1		09/10/2019	Older
7	221	parity_3	10	1	1	0,8		09/10/2019	Older
7	222	parity_3	10	2	2	1,4		09/10/2019	Older
7	223	parity_2	10	1	1	1,3		09/10/2019	Second
7	224	parity_1	10	1	1	0,8		09/10/2019	First
7	225	parity_1	10	1	1	0,7		09/10/2019	First
7	226	parity_1	10	1	1	0,8		09/10/2019	First
7	227	parity_1	10	1	1	0,9		09/10/2019	First
11	228	parity_3	10	1	1	1,8		10/10/2019	Older

11	229	parity_7	10	1	1	0,4		10/10/2019	Older
11	230	parity_3	10	1	1	1,5		10/10/2019	Older
11	231	parity_3	10	1	1	1,1		10/10/2019	Older
11	232	parity_3	10	1	1	1,5		10/10/2019	Older
11	233	parity_3	11	2	1	1,6		10/10/2019	Older
11	234	parity_2	10	1	1	1,8		10/10/2019	Second
11	235	parity_2	11	1	1	1,6		10/10/2019	Second
11	236	parity_2	10	1	1	1,1		10/10/2019	Second
11	237	parity_1	10	1	NA	0,9	IU	10/10/2019	First
11	238	parity_1	12	1	1	1,1		10/10/2019	First
11	239	parity_1	10	1	1	1		10/10/2019	First
11	240	parity_1	10	1	1	1,2		10/10/2019	First
11	241	parity_1	10	1	1	1		10/10/2019	First
11	242	parity_1	10	1	1	1,4		10/10/2019	First
11	243	parity_1	10	2	1	0,8		10/10/2019	First
13	244	parity_4	10	1	1	1,1		11/10/2019	Older
13	245	parity_3	10	1	1	1,1		11/10/2019	Older
13	246	parity_2	10	1	NA	1,4	B	11/10/2019	Second
13	247	parity_1	10	1	1	1,1		11/10/2019	First
13	248	parity_1	10	1	1	0,7		11/10/2019	First
1	249	parity_2	10	1	1	1,1		14/10/2019	Second
1	250	parity_3	10	2	NA	1,3	B	14/10/2019	Older
1	251	parity_1	10	1	1	1,2		14/10/2019	First
1	252	parity_5	10	1	NA	1,3	IU	14/10/2019	Older
1	253	parity_3	10	NA	1	1,6	IU	14/10/2019	Older
1	254	parity_1	10	1	1	1		14/10/2019	First
2	255	parity_5	10	1	NA	1,5	IU	14/10/2019	Older
2	256	parity_1	10	1	1	1,2		14/10/2019	First
2	257	parity_2	10	1	NA	1,2	IU	14/10/2019	Second
2	258	parity_1	10	1	1	1		14/10/2019	First
2	259	parity_1	10	1	1	0,4		14/10/2019	First
2	260	parity_2	10	1	NA	1	B	14/10/2019	Second
2	261	parity_1	10	1	1	1,3		14/10/2019	First
3	262	parity_3	11	NA	2	1,9	IU	14/10/2019	Older
3	263	parity_1	10	1	1	0,7		14/10/2019	First
3	264	parity_2	10	1	NA	0,9	B	14/10/2019	Second
3	265	parity_4	12	3	3	2,4		14/10/2019	Older
3	266	parity_3	10	1	NA	1,5	IU	14/10/2019	Older
3	267	parity_1	10	1	2	1		14/10/2019	First

4	268	parity_4	10	NA	NA	2	B	15/10/2019	Older
4	269	parity_3	10	1	2	1		15/10/2019	Older
4	270	parity_3	10	2	1	2,1		15/10/2019	Older
4	271	parity_3	10	2	2	1,9		15/10/2019	Older
4	272	parity_3	10	NA	1	1,3	IU	15/10/2019	Older
4	273	parity_2	10	1	1	1,1		15/10/2019	Second
4	274	parity_2	10	2	2	1,3		15/10/2019	Second
4	275	parity_2	10	1	1	1,9		15/10/2019	Second
4	276	parity_2	10	1	1	1,6		15/10/2019	Second
4	277	parity_2	10	1	1	1,1		15/10/2019	Second
4	278	parity_2	10	3	3	1,7		15/10/2019	Second
4	279	parity_1	10	NA	2	1,2	IU	15/10/2019	First
4	280	parity_1	10	1	1	1,2		15/10/2019	First
12	281	parity_1	10	1	1	0,7		15/10/2019	First
12	282	parity_5	10	2	2	1,5		15/10/2019	Older
12	283	parity_4	10	2	NA	1,4	S	15/10/2019	Older
12	284	parity_3	10	2	2	1,2		15/10/2019	Older
12	285	parity_3	11	1	1	1,2		15/10/2019	Older
12	286	parity_6	10	1	1	1,3		15/10/2019	Older
12	287	parity_1	10	1	1	0,7		15/10/2019	First
12	288	parity_5	10	2	2	1,2		15/10/2019	Older
12	289	parity_2	12	4	4	2,1		15/10/2019	Second
12	290	parity_2	12	1	NA	0,8	U	15/10/2019	Second
12	291	parity_3	10	1	1	1,1		15/10/2019	Older
1	292	parity_5	12	3	3	2,6		21/10/2019	Older
1	293	parity_5	10	1	NA	1,1	B	21/10/2019	Older
1	294	parity_5	10	2	2	1,8		21/10/2019	Older
1	295	parity_3	11	2	2	1,6		21/10/2019	Older
2	296	parity_2	10	2	2	1,1		21/10/2019	Second
2	297	parity_2	10	2	2	1,6		21/10/2019	Second
2	298	parity_4	12	1	NA	1,4	IU	21/10/2019	Older
2	299	parity_4	10	1	1	1,3		21/10/2019	Older
2	300	parity_2	10	2	2	2,1		21/10/2019	Second
2	301	parity_3	10	2	NA	1,6	S	21/10/2019	Older
14	302	parity_3	10	1	1	1		22/10/2019	Older
14	303	parity_3	10	1	1	0,6		22/10/2019	Older
14	304	parity_3	10	1	1	0,8		22/10/2019	Older
14	305	parity_4	10	NA	NA	0,9	IU + S	22/10/2019	Older
14	306	parity_3	10	1	1	1		22/10/2019	Older

14	307	parity_1	12	1	1	0,9			22/10/2019	First
14	308	parity_1	11	1	1	1			22/10/2019	First
10	309	parity_4	12	1	NA	0,9	IU		23/10/2019	Older
10	310	parity_2	10	1	1	0,5			23/10/2019	Second
10	311	parity_2	11	1	1	0,8			23/10/2019	Second
10	312	parity_1	11	1	1	0,6			23/10/2019	First
10	313	parity_1	10	1	1	0,7			23/10/2019	First
10	314	parity_1	10	1	NA	0,8	S		23/10/2019	First
10	315	parity_1	10	1	1	1			23/10/2019	First
10	316	parity_1	10	2	1	0,8			23/10/2019	First
10	317	parity_1	10	1	NA	0,7	S		23/10/2019	First
10	318	parity_1	10	1	NA	0,5	B		23/10/2019	First
10	319	parity_1	10	1	1	0,7			23/10/2019	First
10	320	parity_1	12	1	1	1			23/10/2019	First
9	321	parity_4	10	NA	1	1,3	B		24/10/2019	Older
9	322	parity_1	10	1	1	0,7			24/10/2019	First
9	323	parity_1	NA	1	1	0,7	S		24/10/2019	First
9	324	parity_1	10	1	1	0,6			24/10/2019	First
9	325	parity_1	10	1	1	0,7			24/10/2019	First
9	326	parity_1	10	2	NA	0,9	B		24/10/2019	First
9	327	parity_2	10	1	1	1,5			24/10/2019	Second
11	328	parity_1	10	2	2	1,7			24/10/2019	First
11	329	parity_1	10	1	NA	0,9	S		24/10/2019	First
11	330	parity_1	10	1	1	0,8			24/10/2019	First
11	331	parity_1	10	NA	1	1,1	IU		24/10/2019	First
11	332	parity_7	12	5	NA	3,2	B		24/10/2019	Older
11	333	parity_4	10	1	1	1,4			24/10/2019	Older
11	334	parity_3	10	1	NA	1,3	IU		24/10/2019	Older
11	335	parity_3	10	1	1	1,3			24/10/2019	Older
11	336	parity_3	10	1	1	1,3			24/10/2019	Older
11	337	parity_2	11	2	2	1,6			24/10/2019	Second
11	338	parity_2	12	2	2	1,8			24/10/2019	Second
11	339	parity_1	10	1	1	1,2			24/10/2019	First
11	340	parity_1	10	2	NA	1,7	B		24/10/2019	First
11	341	parity_1	10	1	1	1,1			24/10/2019	First
11	342	parity_1	10	1	1	1,4			24/10/2019	First
7	343	parity_1	10	1	NA	0,9	B		24/10/2019	First
7	344	parity_3	10	1	1	0,7			24/10/2019	Older
7	345	parity_3	10	3	3	1,5			24/10/2019	Older

7	346	parity_2	10	1	1	1,1		24/10/2019	Second
7	347	parity_4	12	2	2	1,9		24/10/2019	Older
7	348	parity_2	10	2	2	1,7		24/10/2019	Second
4	349	parity_2	10	1	1	1,5		28/10/2019	Second
4	350	parity_2	10	1	1	1,4		28/10/2019	Second
4	351	parity_2	10	1	1	1,8		28/10/2019	Second
4	352	parity_2	10	1	1	1,4		28/10/2019	Second
4	353	parity_2	10	1	1	1,9		28/10/2019	Second
4	354	parity_2	10	1	1	1,7		28/10/2019	Second
4	355	parity_1	10	2	2	1,8		28/10/2019	First
4	356	parity_1	10	1	1	1,4		28/10/2019	First
4	357	parity_1	12	1	1	1		28/10/2019	First
4	358	parity_1	10	1	1	1,9		28/10/2019	First
4	359	parity_1	10	1	NA	1	IU	28/10/2019	First
4	360	parity_3	10	2	2	1,8		28/10/2019	Older
4	361	parity_3	10	2	2	1,8		28/10/2019	Older
4	362	parity_3	10	1	1	1,4		28/10/2019	Older
4	363	parity_3	10	1	1	1,8		28/10/2019	Older
4	364	parity_3	10	3	3	2,1		28/10/2019	Older
4	365	parity_3	10	1	1	2		28/10/2019	Older
4	366	parity_3	10	1	NA	0,9	B	28/10/2019	Older
4	367	parity_2	10	1	1	2,2		28/10/2019	Second
4	368	parity_2	10	1	1	1,2		28/10/2019	Second
14	369	parity_3	10	1	NA	1	S	29/10/2019	Older
14	370	parity_5	10	1	NA	1,2	S	29/10/2019	Older
14	371	parity_2	10	1	1	1,3		29/10/2019	Second
3	372	parity_1	12	1	1	1,2		30/10/2019	First
3	373	parity_4	12	3	3	3,2		30/10/2019	Older
3	374	parity_2	10	1	NA	1,7	IU	30/10/2019	Second
3	375	parity_2	10	1	NA	1	IU	30/10/2019	Second
3	376	parity_1	10	1	1	1,2		30/10/2019	First
3	377	parity_1	10	1	NA	1,8	IU	30/10/2019	First
3	378	parity_2	10	1	NA	1	S	30/10/2019	Second
3	379	parity_1	10	1	1	1,4		30/10/2019	First
3	380	parity_3	10	1	1	1,8		30/10/2019	Older
3	381	parity_1	10	1	NA	0,9	S	30/10/2019	First
10	382	parity_4	12	2	NA	1,1	B	30/10/2019	Older
10	383	parity_2	10	1	NA	1	B	30/10/2019	Second
10	384	parity_1	10	1	NA	0,4	S	30/10/2019	First

10	385	parity_2	10	1	1	1		30/10/2019	Second
10	386	parity_1	10	2	NA	0,9	S + blod	30/10/2019	First
7	387	parity_5	10	2	2	2		30/10/2019	Older
7	388	parity_5	10	1	1	1,4		30/10/2019	Older
7	389	parity_2	10	2	1	1,5		30/10/2019	Second
7	390	parity_1	10	2	2	1,7		30/10/2019	First
7	391	parity_1	10	1	1	1,3		30/10/2019	First
11	392	parity_1	10	1	1	0,9		31/10/2019	First
11	393	parity_1	12	1	1	1,2		31/10/2019	First
11	394	parity_1	10	1	1	1,4		31/10/2019	First
11	395	parity_1	10	2	1	1		31/10/2019	First
11	396	parity_1	10	1	1	1,1		31/10/2019	First
11	397	parity_4	13	1	1	1,1		31/10/2019	Older
11	398	parity_5	12	1	NA	1,7	B	31/10/2019	Older
11	399	parity_1	10	1	1	1,2		31/10/2019	First
11	400	parity_1	10	1	1	1,5		31/10/2019	First
11	401	parity_3	10	1	1	0,9		31/10/2019	Older
11	402	parity_3	12	2	2	1,9		31/10/2019	Older
11	403	parity_3	10	1	1	1,4		31/10/2019	Older
11	404	parity_4	13	3	NA	2,7	IU + behandlet	31/10/2019	Older
9	405	parity_2	10	1	NA	1,4	S	31/10/2019	Second
9	406	parity_6	10	1	1	1,5		31/10/2019	Older
9	407	parity_4	11	2	NA	1,2	B	31/10/2019	Older
9	408	parity_2	10	NA	1	0,9	IU	31/10/2019	Second
9	409	parity_2	12	1	NA	1,7	S	31/10/2019	Second
9	410	parity_1	10	1	NA	1	S	31/10/2019	First
2	411	parity_2	10	1	1	1,3		04/11/2019	Second
2	412	parity_3	10	1	NA	0,7	IU	04/11/2019	Older
1	413	parity_1	10	1	1	0,8		04/11/2019	First
1	414	parity_3	10	2	2	1,4		04/11/2019	Older
1	415	parity_3	12	2	NA	1,9	S	04/11/2019	Older
1	416	parity_3	10	1	1	1,8		04/11/2019	Older
1	417	parity_1	10	2	NA	2	S	04/11/2019	First
1	418	parity_2	12	2	2	1,8		04/11/2019	Second
1	419	parity_1	10	1	NA	1,5	IU	04/11/2019	First
4	420	parity_7	11	2	1	0,9		05/11/2019	Older
4	421	parity_2	10	1	1	0,7		05/11/2019	Second
4	422	parity_3	10	1	1	0,8		05/11/2019	Older
4	423	parity_4	10	1	1	1,2		05/11/2019	Older

4	424	parity_3	10	1	1	0,7		05/11/2019	Older
4	425	parity_3	10	2	NA	1,6	B	05/11/2019	Older
4	426	parity_2	10	2	2	1,3		05/11/2019	Second
4	427	parity_3	10	2	2	1,4		05/11/2019	Older
4	428	parity_3	10	1	1	0,9		05/11/2019	Older
4	429	parity_2	10	1	1	1,5		05/11/2019	Second
4	430	parity_2	10	1	1	1,1		05/11/2019	Second
4	431	parity_3	10	2	2	1,6		05/11/2019	Older
7	432	parity_5	10	1	NA	1,2	D	06/11/2019	Older
7	433	parity_4	10	1	1	1,7		06/11/2019	Older
7	434	parity_3	11	2	2	2,2		06/11/2019	Older
7	435	parity_2	12	1	1	1,9		06/11/2019	Second
7	436	parity_2	10	NA	1	1,3	IU	06/11/2019	Second
7	437	parity_2	10	1	NA	0,9	S	06/11/2019	Second
7	438	parity_2	10	1	1	1,3		06/11/2019	Second
7	439	parity_5	14	1	1	0,9		06/11/2019	Older
7	440	parity_1	10	1	NA	1,4	S	06/11/2019	First
13	441	parity_4	10	1	1	1,2		07/11/2019	Older
13	442	parity_3	10	2	2	1,3		07/11/2019	Older
13	443	parity_4	10	1	NA	0,9	B	07/11/2019	Older
13	444	parity_6	10	1	1	0,8		07/11/2019	Older
13	445	parity_1	10	1	1	0,6		07/11/2019	First
13	446	parity_1	10	1	1	0,7		07/11/2019	First
11	447	parity_6	10	1	1	1,1		07/11/2019	Older
11	448	parity_3	10	1	1	0,5		07/11/2019	Older
11	449	parity_3	10	1	NA	0,4	S	07/11/2019	Older
11	450	parity_2	10	1	1	0,7		07/11/2019	Second
11	451	parity_2	10	1	1	0,5		07/11/2019	Second
11	452	parity_2	10	1	1	0,6		07/11/2019	Second
11	453	parity_2	10	1	1	0,5		07/11/2019	Second
11	454	parity_2	10	2	2	0,8		07/11/2019	Second
11	455	parity_1	12	1	NA	0,4	S	07/11/2019	First
2	456	parity_5	10	1	NA	0,8	IU	11/11/2019	Older
2	457	parity_2	10	NA	2	1,5	IU	11/11/2019	Second
2	458	parity_6	10	1	1	0,8		11/11/2019	Older
2	459	parity_6	10	1	NA	1	IU	11/11/2019	Older
2	460	parity_10	10	NA	1	1,4	IU	11/11/2019	Older
2	461	parity_4	10	1	1	0,9		11/11/2019	Older
2	462	parity_1	10	2	2	0,9		11/11/2019	First

1	463	parity_6	10	1	1	1		11/11/2019	Older
1	464	parity_5	13	3	NA	1,6	IU	11/11/2019	Older
1	465	parity_3	10	1	1	1		11/11/2019	Older
1	466	parity_2	10	NA	1	1	IU	11/11/2019	Second
1	467	parity_5	10	2	NA	1,2	S	11/11/2019	Older
1	468	parity_5	10	1	NA	0,8	B	11/11/2019	Older
3	469	parity_4	10	1	1	0,9		11/11/2019	Older
3	470	parity_3	10	1	1	1,1		11/11/2019	Older
3	471	parity_3	10	1	1	0,8		11/11/2019	Older
3	472	parity_1	10	NA	NA	0,6	IU + B	11/11/2019	First
4	473	parity_1	10	2	NA	0,9	B	12/11/2019	First
4	474	parity_2	10	2	2	1		12/11/2019	Second
4	475	parity_1	12	2	2	1,3		12/11/2019	First
4	476	parity_3	10	2	2	1,3		12/11/2019	Older
4	477	parity_1	10	3	NA	1,7	B	12/11/2019	First
4	478	parity_3	10	3	3	1,6		12/11/2019	Older
4	479	parity_2	11	1	NA	0,8	B	12/11/2019	Second
4	480	parity_1	12	1	1	0,8		12/11/2019	First
4	481	parity_6	13	1	1	0,9		12/11/2019	Older
11	482	parity_5	10	1	1	1,1		14/11/2019	Older
11	483	parity_5	10	1	1	0,6		14/11/2019	Older
11	484	parity_3	10	1	1	0,6		14/11/2019	Older
11	485	parity_2	10	1	NA	0,1	S	14/11/2019	Second
11	486	parity_2	10	1	1	0,7		14/11/2019	Second
11	487	parity_2	10	1	1	0,6		14/11/2019	Second
11	488	parity_1	10	1	1	0,4		14/11/2019	First

A-2. Complete dataset without missing observations

Table B. Complete dataset after the missing observations has been deleted. Yellow row = Shift to D2 device.

Herd id	Cow id	Parity	Test 1	Test 2	Test 3	Test 4	Error code	Date	Parity Group
1	1	parity_4	12	2	2	1,5	NA	16/09/2019	Older
1	3	parity_1	10	1	1	1,1	NA	16/09/2019	First
2	5	parity_2	12	1	1	1,1	NA	16/09/2019	Second
2	7	parity_4	10	1	1	0,8	NA	16/09/2019	Older
2	8	parity_1	10	1	1	0,8	NA	16/09/2019	First
2	9	parity_1	10	1	1	0,9	NA	16/09/2019	First
2	10	parity_3	10	1	1	1,3	NA	16/09/2019	Older
3	11	parity_5	10	1	1	1,1	NA	16/09/2019	Older
3	12	parity_3	12	4	4	2,2	NA	16/09/2019	Older

3	13	parity_2	10	1	1	0,9	NA		16/09/2019	Second
3	14	parity_2	10	1	1	1,3	NA		16/09/2019	Second
3	15	parity_1	10	1	1	1	NA		16/09/2019	First
4	16	parity_3	13	2	2	1,3	NA		17/09/2019	Older
4	17	parity_1	12	4	4	1,6	NA		17/09/2019	First
4	19	parity_3	10	2	2	1,8	NA		17/09/2019	Older
4	21	parity_2	10	1	1	1	NA		17/09/2019	Second
4	22	parity_8	10	1	1	1,5	NA		17/09/2019	Older
5	23	parity_3	10	1	1	1,7	NA		17/09/2019	Older
5	24	parity_3	10	1	1	0,9	NA		17/09/2019	Older
5	25	parity_2	10	1	1	1	NA		17/09/2019	Second
5	26	parity_5	10	2	1	1,8	NA		17/09/2019	Older
5	27	parity_4	10	1	1	1,8	NA		17/09/2019	Older
6	28	parity_4	12	1	1	1,3	NA		18/09/2019	Older
6	30	parity_4	10	2	1	0,9	NA		18/09/2019	Older
7	31	parity_3	10	1	1	0,8	NA		18/09/2019	Older
7	33	parity_3	10	2	2	1,4	NA		18/09/2019	Older
7	35	parity_1	10	1	1	1,2	NA		18/09/2019	First
7	36	parity_2	10	1	1	0,7	NA		18/09/2019	Second
7	37	parity_3	10	1	1	0,8	NA		18/09/2019	Older
8	40	parity_1	10	1	1	0,8	NA		18/09/2019	First
8	41	parity_1	10	1	1	0,5	NA		18/09/2019	First
8	42	parity_2	12	1	2	1,5	NA		18/09/2019	Second
8	43	parity_1	10	1	1	0,8	NA		18/09/2019	First
8	44	parity_3	12	3	3	2,1	NA		18/09/2019	Older
8	45	parity_2	10	1	1	1,6	NA		18/09/2019	Second
8	46	parity_2	10	1	1	0,8	NA		18/09/2019	Second
9	47	parity_3	10	1	1	1,6	NA		19/09/2019	Older
9	48	parity_3	10	1	1	1,1	NA		19/09/2019	Older
9	49	parity_2	10	1	1	1,4	NA		19/09/2019	Second
9	50	parity_3	10	1	1	1,5	NA		19/09/2019	Older
9	51	parity_2	10	2	2	1,3	NA		19/09/2019	Second
9	52	parity_2	10	1	1	1,6	NA		19/09/2019	Second
9	54	parity_2	12	1	1	1,2	NA		19/09/2019	Second
2	55	parity_1	10	1	1	1,1	NA		23/09/2019	First
2	56	parity_3	10	1	1	0,9	NA		23/09/2019	Older
2	57	parity_2	10	1	1	1	NA		23/09/2019	Second
2	58	parity_2	10	1	1	1,1	NA		23/09/2019	Second
1	60	parity_1	13	1	1	1,1	NA		23/09/2019	First
1	62	parity_1	10	1	1	0,8	NA		23/09/2019	First
1	64	parity_2	10	1	1	1,2	NA		23/09/2019	Second
1	66	parity_2	10	1	1	1,2	NA		23/09/2019	Second
1	67	parity_2	12	2	2	1,3	NA		23/09/2019	Second
4	69	parity_1	10	1	1	0,7	NA		24/09/2019	First

4	70	parity_1	13	1	1	1,3	NA	24/09/2019	First
4	71	parity_3	12	4	4	2,6	NA	24/09/2019	Older
4	72	parity_1	10	1	1	1,3	NA	24/09/2019	First
4	73	parity_3	10	2	2	1,6	NA	24/09/2019	Older
4	74	parity_3	10	1	1	0,8	NA	24/09/2019	Older
4	75	parity_1	10	1	1	1,1	NA	24/09/2019	First
5	77	parity_3	10	1	1	1,3	NA	24/09/2019	Older
5	78	parity_1	12	2	1	1,1	NA	24/09/2019	First
10	79	parity_1	10	1	1	0,8	NA	25/09/2019	First
10	80	parity_3	12	1	1	1	NA	25/09/2019	Older
10	81	parity_2	10	1	1	0,7	NA	25/09/2019	Second
10	82	parity_1	10	1	1	1,2	NA	25/09/2019	First
10	84	parity_4	10	1	1	0,7	NA	25/09/2019	Older
10	86	parity_2	12	4	4	1,8	NA	25/09/2019	Second
10	88	parity_2	10	1	1	1	NA	25/09/2019	Second
10	90	parity_1	10	1	1	0,8	NA	25/09/2019	First
10	93	parity_1	10	1	1	0,4	NA	25/09/2019	First
10	94	parity_1	10	1	1	0,9	NA	25/09/2019	First
10	95	parity_2	10	1	1	1,4	NA	25/09/2019	Second
10	96	parity_2	10	2	2	1,1	NA	25/09/2019	Second
11	97	parity_3	10	1	1	1,2	NA	26/09/2019	Older
11	98	parity_4	12	1	1	1,2	NA	26/09/2019	Older
11	99	parity_3	12	5	5	4,2	NA	26/09/2019	Older
11	100	parity_3	10	1	1	0,6	NA	26/09/2019	Older
11	101	parity_2	10	2	1	0,7	NA	26/09/2019	Second
11	102	parity_2	10	1	1	0,5	NA	26/09/2019	Second
11	103	parity_1	10	1	1	0,6	NA	26/09/2019	First
11	104	parity_1	10	1	1	1,1	NA	26/09/2019	First
11	105	parity_1	10	1	1	0,5	NA	26/09/2019	First
11	106	parity_1	10	1	1	0,5	NA	26/09/2019	First
11	107	parity_1	10	1	1	0,9	NA	26/09/2019	First
9	110	parity_5	14	1	1	1,5	NA	26/09/2019	Older
9	115	parity_2	10	1	2	1	NA	26/09/2019	Second
2	116	parity_1	10	1	1	1	NA	30/09/2019	First
2	117	parity_4	12	1	1	1	NA	30/09/2019	Older
2	118	parity_1	10	1	1	1	NA	30/09/2019	First
2	119	parity_4	10	1	1	1,4	NA	30/09/2019	Older
2	120	parity_2	10	1	1	1,4	NA	30/09/2019	Second
1	122	parity_3	10	1	1	0,8	NA	30/09/2019	Older
1	124	parity_3	10	1	1	0,7	NA	30/09/2019	Older
3	127	parity_4	12	2	2	1,9	NA	30/09/2019	Older
3	128	parity_2	12	1	1	1,4	NA	30/09/2019	Second
3	129	parity_2	12	1	1	1,1	NA	30/09/2019	Second
3	130	parity_2	10	3	2	1,6	NA	30/09/2019	Second

3	131	parity_2	10	1	1	1,4	NA	30/09/2019	Second
3	132	parity_1	10	1	1	1,1	NA	30/09/2019	First
4	133	parity_3	10	1	1	0,7	NA	01/10/2019	Older
4	134	parity_4	12	2	1	2	NA	01/10/2019	Older
4	136	parity_2	10	2	2	1,6	NA	01/10/2019	Second
4	137	parity_2	10	1	1	1,4	NA	01/10/2019	Second
4	139	parity_2	10	2	2	2,2	NA	01/10/2019	Second
4	140	parity_2	10	1	1	1,6	NA	01/10/2019	Second
4	141	parity_2	10	1	1	1	NA	01/10/2019	Second
4	142	parity_1	10	1	1	1,4	NA	01/10/2019	First
4	143	parity_3	10	2	2	2,1	NA	01/10/2019	Older
4	144	parity_1	10	1	1	1	NA	01/10/2019	First
4	145	parity_1	10	1	1	1,3	NA	01/10/2019	First
4	146	parity_1	10	1	1	0,9	NA	01/10/2019	First
4	147	parity_1	10	1	1	1,1	NA	01/10/2019	First
4	148	parity_1	10	1	1	1,8	NA	01/10/2019	First
12	151	parity_3	10	1	1	1,3	NA	01/10/2019	Older
12	152	parity_1	10	1	2	1,2	NA	01/10/2019	First
12	153	parity_2	12	1	1	1,2	NA	01/10/2019	Second
12	154	parity_4	13	1	1	1	NA	01/10/2019	Older
12	155	parity_3	11	1	2	0,9	NA	01/10/2019	Older
12	158	parity_4	10	1	1	1,6	NA	01/10/2019	Older
12	159	parity_4	10	1	1	1,4	NA	01/10/2019	Older
12	160	parity_4	10	1	1	1,6	NA	01/10/2019	Older
7	161	parity_5	10	1	1	1,1	NA	02/10/2019	Older
7	162	parity_5	10	1	1	1,6	NA	02/10/2019	Older
7	164	parity_5	10	1	1	1,7	NA	02/10/2019	Older
7	166	parity_3	10	1	1	1	NA	02/10/2019	Older
7	167	parity_2	10	1	1	1	NA	02/10/2019	Second
7	168	parity_2	10	1	1	1,4	NA	02/10/2019	Second
11	169	parity_1	12	2	1	0,8	NA	03/10/2019	First
11	170	parity_3	10	1	1	0,7	NA	03/10/2019	Older
11	172	parity_1	10	1	1	0,8	NA	03/10/2019	First
11	173	parity_4	10	1	1	1,2	NA	03/10/2019	Older
11	174	parity_3	10	1	1	1,3	NA	03/10/2019	Older
11	175	parity_3	10	1	1	0,6	NA	03/10/2019	Older
11	176	parity_3	12	4	3	2,3	NA	03/10/2019	Older
11	177	parity_2	10	1	1	1	NA	03/10/2019	Second
13	179	parity_4	10	1	1	1	NA	04/10/2019	Older
13	180	parity_4	10	1	1	1,3	NA	04/10/2019	Older
13	181	parity_4	10	1	1	1	NA	04/10/2019	Older
13	182	parity_1	10	2	1	1,2	NA	04/10/2019	First
13	183	parity_1	10	1	1	0,9	NA	04/10/2019	First
1	185	parity_1	10	1	1	1,7	NA	07/10/2019	First

2	188	parity_1	13	1	1	1	NA	07/10/2019	First
2	189	parity_2	10	1	1	1,5	NA	07/10/2019	Second
2	190	parity_5	10	1	1	1,4	NA	07/10/2019	Older
4	191	parity_5	10	1	1	1,3	NA	08/10/2019	Older
4	192	parity_4	11	2	2	1,1	NA	08/10/2019	Older
4	193	parity_4	10	1	1	1,6	NA	08/10/2019	Older
4	194	parity_4	11	2	2	2,3	NA	08/10/2019	Older
4	195	parity_4	10	1	1	1,6	NA	08/10/2019	Older
4	196	parity_3	10	2	2	1,5	NA	08/10/2019	Older
4	197	parity_3	10	1	1	1,5	NA	08/10/2019	Older
4	198	parity_2	10	1	1	1,1	NA	08/10/2019	Second
4	199	parity_1	10	1	1	1,1	NA	08/10/2019	First
4	200	parity_1	10	1	1	1,6	NA	08/10/2019	First
4	201	parity_2	13	1	1	1,5	NA	08/10/2019	Second
4	202	parity_2	10	1	1	0,5	NA	08/10/2019	Second
4	203	parity_2	11	3	3	2,2	NA	08/10/2019	Second
4	204	parity_2	10	1	1	1,3	NA	08/10/2019	Second
4	205	parity_2	10	1	1	1,4	NA	08/10/2019	Second
4	206	parity_2	10	1	1	1,4	NA	08/10/2019	Second
4	207	parity_2	10	1	1	1,1	NA	08/10/2019	Second
4	208	parity_2	10	1	1	1,4	NA	08/10/2019	Second
4	209	parity_1	10	1	1	1,1	NA	08/10/2019	First
4	210	parity_1	10	1	1	0,9	NA	08/10/2019	First
10	211	parity_3	10	1	1	0,8	NA	09/10/2019	Older
10	212	parity_2	10	1	1	1,1	NA	09/10/2019	Second
10	213	parity_1	10	1	1	0,8	NA	09/10/2019	First
10	214	parity_1	10	1	1	0,8	NA	09/10/2019	First
10	215	parity_4	10	2	2	1,5	NA	09/10/2019	Older
10	216	parity_1	10	1	1	1,2	NA	09/10/2019	First
10	219	parity_5	10	1	1	0,8	NA	09/10/2019	Older
7	220	parity_4	10	1	2	1,1	NA	09/10/2019	Older
7	221	parity_3	10	1	1	0,8	NA	09/10/2019	Older
7	222	parity_3	10	2	2	1,4	NA	09/10/2019	Older
7	223	parity_2	10	1	1	1,3	NA	09/10/2019	Second
7	224	parity_1	10	1	1	0,8	NA	09/10/2019	First
7	225	parity_1	10	1	1	0,7	NA	09/10/2019	First
7	226	parity_1	10	1	1	0,8	NA	09/10/2019	First
7	227	parity_1	10	1	1	0,9	NA	09/10/2019	First
11	228	parity_3	10	1	1	1,8	NA	10/10/2019	Older
11	229	parity_7	10	1	1	0,4	NA	10/10/2019	Older
11	230	parity_3	10	1	1	1,5	NA	10/10/2019	Older
11	231	parity_3	10	1	1	1,1	NA	10/10/2019	Older
11	232	parity_3	10	1	1	1,5	NA	10/10/2019	Older
11	233	parity_3	11	2	1	1,6	NA	10/10/2019	Older

11	234	parity_2	10	1	1	1,8	NA	10/10/2019	Second
11	235	parity_2	11	1	1	1,6	NA	10/10/2019	Second
11	236	parity_2	10	1	1	1,1	NA	10/10/2019	Second
11	238	parity_1	12	1	1	1,1	NA	10/10/2019	First
11	239	parity_1	10	1	1	1	NA	10/10/2019	First
11	240	parity_1	10	1	1	1,2	NA	10/10/2019	First
11	241	parity_1	10	1	1	1	NA	10/10/2019	First
11	242	parity_1	10	1	1	1,4	NA	10/10/2019	First
11	243	parity_1	10	2	1	0,8	NA	10/10/2019	First
13	244	parity_4	10	1	1	1,1	NA	11/10/2019	Older
13	245	parity_3	10	1	1	1,1	NA	11/10/2019	Older
13	247	parity_1	10	1	1	1,1	NA	11/10/2019	First
13	248	parity_1	10	1	1	0,7	NA	11/10/2019	First
1	249	parity_2	10	1	1	1,1	NA	14/10/2019	Second
1	251	parity_1	10	1	1	1,2	NA	14/10/2019	First
1	254	parity_1	10	1	1	1	NA	14/10/2019	First
2	256	parity_1	10	1	1	1,2	NA	14/10/2019	First
2	258	parity_1	10	1	1	1	NA	14/10/2019	First
2	259	parity_1	10	1	1	0,4	NA	14/10/2019	First
2	261	parity_1	10	1	1	1,3	NA	14/10/2019	First
3	263	parity_1	10	1	1	0,7	NA	14/10/2019	First
3	265	parity_4	12	3	3	2,4	NA	14/10/2019	Older
3	267	parity_1	10	1	2	1	NA	14/10/2019	First
4	269	parity_3	10	1	2	1	NA	15/10/2019	Older
4	270	parity_3	10	2	1	2,1	NA	15/10/2019	Older
4	271	parity_3	10	2	2	1,9	NA	15/10/2019	Older
4	273	parity_2	10	1	1	1,1	NA	15/10/2019	Second
4	274	parity_2	10	2	2	1,3	NA	15/10/2019	Second
4	275	parity_2	10	1	1	1,9	NA	15/10/2019	Second
4	276	parity_2	10	1	1	1,6	NA	15/10/2019	Second
4	277	parity_2	10	1	1	1,1	NA	15/10/2019	Second
4	278	parity_2	10	3	3	1,7	NA	15/10/2019	Second
4	280	parity_1	10	1	1	1,2	NA	15/10/2019	First
12	281	parity_1	10	1	1	0,7	NA	15/10/2019	First
12	282	parity_5	10	2	2	1,5	NA	15/10/2019	Older
12	284	parity_3	10	2	2	1,2	NA	15/10/2019	Older
12	285	parity_3	11	1	1	1,2	NA	15/10/2019	Older
12	286	parity_6	10	1	1	1,3	NA	15/10/2019	Older
12	287	parity_1	10	1	1	0,7	NA	15/10/2019	First
12	288	parity_5	10	2	2	1,2	NA	15/10/2019	Older
12	289	parity_2	12	4	4	2,1	NA	15/10/2019	Second
12	291	parity_3	10	1	1	1,1	NA	15/10/2019	Older
1	292	parity_5	12	3	3	2,6	NA	21/10/2019	Older
1	294	parity_5	10	2	2	1,8	NA	21/10/2019	Older

1	295	parity_3	11	2	2	1,6	NA	21/10/2019	Older
2	296	parity_2	10	2	2	1,1	NA	21/10/2019	Second
2	297	parity_2	10	2	2	1,6	NA	21/10/2019	Second
2	299	parity_4	10	1	1	1,3	NA	21/10/2019	Older
2	300	parity_2	10	2	2	2,1	NA	21/10/2019	Second
14	302	parity_3	10	1	1	1	NA	22/10/2019	Older
14	303	parity_3	10	1	1	0,6	NA	22/10/2019	Older
14	304	parity_3	10	1	1	0,8	NA	22/10/2019	Older
14	306	parity_3	10	1	1	1	NA	22/10/2019	Older
14	307	parity_1	12	1	1	0,9	NA	22/10/2019	First
14	308	parity_1	11	1	1	1	NA	22/10/2019	First
10	310	parity_2	10	1	1	0,5	NA	23/10/2019	Second
10	311	parity_2	11	1	1	0,8	NA	23/10/2019	Second
10	312	parity_1	11	1	1	0,6	NA	23/10/2019	First
10	313	parity_1	10	1	1	0,7	NA	23/10/2019	First
10	315	parity_1	10	1	1	1	NA	23/10/2019	First
10	316	parity_1	10	2	1	0,8	NA	23/10/2019	First
10	319	parity_1	10	1	1	0,7	NA	23/10/2019	First
10	320	parity_1	12	1	1	1	NA	23/10/2019	First
9	322	parity_1	10	1	1	0,7	NA	24/10/2019	First
9	324	parity_1	10	1	1	0,6	NA	24/10/2019	First
9	325	parity_1	10	1	1	0,7	NA	24/10/2019	First
9	327	parity_2	10	1	1	1,5	NA	24/10/2019	Second
11	328	parity_1	10	2	2	1,7	NA	24/10/2019	First
11	330	parity_1	10	1	1	0,8	NA	24/10/2019	First
11	333	parity_4	10	1	1	1,4	NA	24/10/2019	Older
11	335	parity_3	10	1	1	1,3	NA	24/10/2019	Older
11	336	parity_3	10	1	1	1,3	NA	24/10/2019	Older
11	337	parity_2	11	2	2	1,6	NA	24/10/2019	Second
11	338	parity_2	12	2	2	1,8	NA	24/10/2019	Second
11	339	parity_1	10	1	1	1,2	NA	24/10/2019	First
11	341	parity_1	10	1	1	1,1	NA	24/10/2019	First
11	342	parity_1	10	1	1	1,4	NA	24/10/2019	First
7	344	parity_3	10	1	1	0,7	NA	24/10/2019	Older
7	345	parity_3	10	3	3	1,5	NA	24/10/2019	Older
7	346	parity_2	10	1	1	1,1	NA	24/10/2019	Second
7	347	parity_4	12	2	2	1,9	NA	24/10/2019	Older
7	348	parity_2	10	2	2	1,7	NA	24/10/2019	Second
4	349	parity_2	10	1	1	1,5	NA	28/10/2019	Second
4	350	parity_2	10	1	1	1,4	NA	28/10/2019	Second
4	351	parity_2	10	1	1	1,8	NA	28/10/2019	Second
4	352	parity_2	10	1	1	1,4	NA	28/10/2019	Second
4	353	parity_2	10	1	1	1,9	NA	28/10/2019	Second
4	354	parity_2	10	1	1	1,7	NA	28/10/2019	Second

4	355	parity_1	10	2	2	1,8	NA	28/10/2019	First
4	356	parity_1	10	1	1	1,4	NA	28/10/2019	First
4	357	parity_1	12	1	1	1	NA	28/10/2019	First
4	358	parity_1	10	1	1	1,9	NA	28/10/2019	First
4	360	parity_3	10	2	2	1,8	NA	28/10/2019	Older
4	361	parity_3	10	2	2	1,8	NA	28/10/2019	Older
4	362	parity_3	10	1	1	1,4	NA	28/10/2019	Older
4	363	parity_3	10	1	1	1,8	NA	28/10/2019	Older
4	364	parity_3	10	3	3	2,1	NA	28/10/2019	Older
4	365	parity_3	10	1	1	2	NA	28/10/2019	Older
4	367	parity_2	10	1	1	2,2	NA	28/10/2019	Second
4	368	parity_2	10	1	1	1,2	NA	28/10/2019	Second
14	371	parity_2	10	1	1	1,3	NA	29/10/2019	Second
3	372	parity_1	12	1	1	1,2	NA	30/10/2019	First
3	373	parity_4	12	3	3	3,2	NA	30/10/2019	Older
3	376	parity_1	10	1	1	1,2	NA	30/10/2019	First
3	379	parity_1	10	1	1	1,4	NA	30/10/2019	First
3	380	parity_3	10	1	1	1,8	NA	30/10/2019	Older
10	385	parity_2	10	1	1	1	NA	30/10/2019	Second
7	387	parity_5	10	2	2	2	NA	30/10/2019	Older
7	388	parity_5	10	1	1	1,4	NA	30/10/2019	Older
7	389	parity_2	10	2	1	1,5	NA	30/10/2019	Second
7	390	parity_1	10	2	2	1,7	NA	30/10/2019	First
7	391	parity_1	10	1	1	1,3	NA	30/10/2019	First
11	392	parity_1	10	1	1	0,9	NA	31/10/2019	First
11	393	parity_1	12	1	1	1,2	NA	31/10/2019	First
11	394	parity_1	10	1	1	1,4	NA	31/10/2019	First
11	395	parity_1	10	2	1	1	NA	31/10/2019	First
11	396	parity_1	10	1	1	1,1	NA	31/10/2019	First
11	397	parity_4	13	1	1	1,1	NA	31/10/2019	Older
11	399	parity_1	10	1	1	1,2	NA	31/10/2019	First
11	400	parity_1	10	1	1	1,5	NA	31/10/2019	First
11	401	parity_3	10	1	1	0,9	NA	31/10/2019	Older
11	402	parity_3	12	2	2	1,9	NA	31/10/2019	Older
11	403	parity_3	10	1	1	1,4	NA	31/10/2019	Older
9	406	parity_6	10	1	1	1,5	NA	31/10/2019	Older
2	411	parity_2	10	1	1	1,3	NA	04/11/2019	Second
1	413	parity_1	10	1	1	0,8	NA	04/11/2019	First
1	414	parity_3	10	2	2	1,4	NA	04/11/2019	Older
1	416	parity_3	10	1	1	1,8	NA	04/11/2019	Older
1	418	parity_2	12	2	2	1,8	NA	04/11/2019	Second
4	420	parity_7	11	2	1	0,9	NA	05/11/2019	Older
4	421	parity_2	10	1	1	0,7	NA	05/11/2019	Second
4	422	parity_3	10	1	1	0,8	NA	05/11/2019	Older

4	423	parity_4	10	1	1	1,2	NA	05/11/2019	Older
4	424	parity_3	10	1	1	0,7	NA	05/11/2019	Older
4	426	parity_2	10	2	2	1,3	NA	05/11/2019	Second
4	427	parity_3	10	2	2	1,4	NA	05/11/2019	Older
4	428	parity_3	10	1	1	0,9	NA	05/11/2019	Older
4	429	parity_2	10	1	1	1,5	NA	05/11/2019	Second
4	430	parity_2	10	1	1	1,1	NA	05/11/2019	Second
4	431	parity_3	10	2	2	1,6	NA	05/11/2019	Older
7	433	parity_4	10	1	1	1,7	NA	06/11/2019	Older
7	434	parity_3	11	2	2	2,2	NA	06/11/2019	Older
7	435	parity_2	12	1	1	1,9	NA	06/11/2019	Second
7	438	parity_2	10	1	1	1,3	NA	06/11/2019	Second
7	439	parity_5	14	1	1	0,9	NA	06/11/2019	Older
13	441	parity_4	10	1	1	1,2	NA	07/11/2019	Older
13	442	parity_3	10	2	2	1,3	NA	07/11/2019	Older
13	444	parity_6	10	1	1	0,8	NA	07/11/2019	Older
13	445	parity_1	10	1	1	0,6	NA	07/11/2019	First
13	446	parity_1	10	1	1	0,7	NA	07/11/2019	First
11	447	parity_6	10	1	1	1,1	NA	07/11/2019	Older
11	448	parity_3	10	1	1	0,5	NA	07/11/2019	Older
11	450	parity_2	10	1	1	0,7	NA	07/11/2019	Second
11	451	parity_2	10	1	1	0,5	NA	07/11/2019	Second
11	452	parity_2	10	1	1	0,6	NA	07/11/2019	Second
11	453	parity_2	10	1	1	0,5	NA	07/11/2019	Second
11	454	parity_2	10	2	2	0,8	NA	07/11/2019	Second
2	458	parity_6	10	1	1	0,8	NA	11/11/2019	Older
2	461	parity_4	10	1	1	0,9	NA	11/11/2019	Older
2	462	parity_1	10	2	2	0,9	NA	11/11/2019	First
1	463	parity_6	10	1	1	1	NA	11/11/2019	Older
1	465	parity_3	10	1	1	1	NA	11/11/2019	Older
3	469	parity_4	10	1	1	0,9	NA	11/11/2019	Older
3	470	parity_3	10	1	1	1,1	NA	11/11/2019	Older
3	471	parity_3	10	1	1	0,8	NA	11/11/2019	Older
4	474	parity_2	10	2	2	1	NA	12/11/2019	Second
4	475	parity_1	12	2	2	1,3	NA	12/11/2019	First
4	476	parity_3	10	2	2	1,3	NA	12/11/2019	Older
4	478	parity_3	10	3	3	1,6	NA	12/11/2019	Older
4	480	parity_1	12	1	1	0,8	NA	12/11/2019	First
4	481	parity_6	13	1	1	0,9	NA	12/11/2019	Older
11	482	parity_5	10	1	1	1,1	NA	14/11/2019	Older
11	483	parity_5	10	1	1	0,6	NA	14/11/2019	Older
11	484	parity_3	10	1	1	0,6	NA	14/11/2019	Older
11	486	parity_2	10	1	1	0,7	NA	14/11/2019	Second
11	487	parity_2	10	1	1	0,6	NA	14/11/2019	Second

11	488	parity_1	10	1	1	0,4	NA	14/11/2019	First

Appendix B – Correction Factor

B-1. Measurements of six blood samples; BHB-check, FSP-Neo & External laboratory

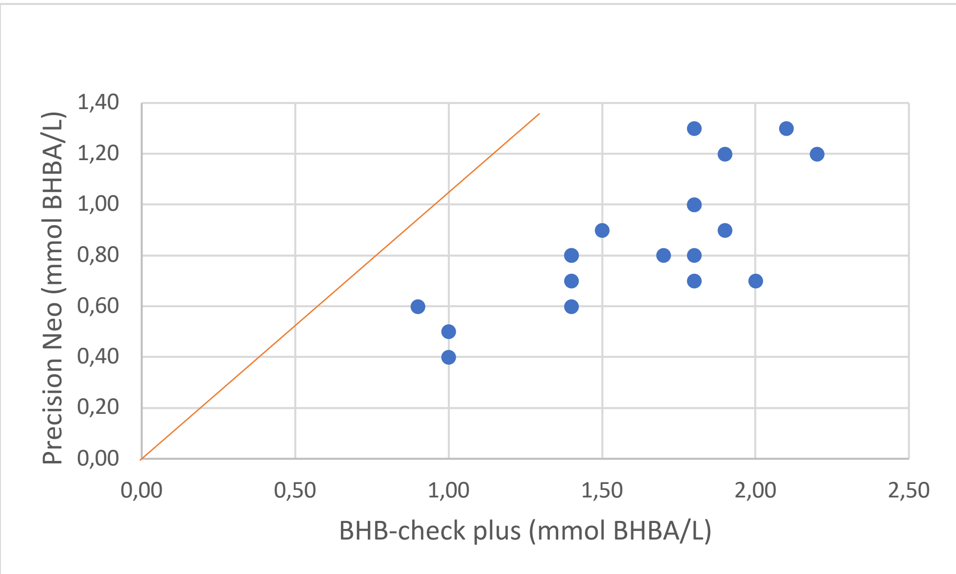
Table C. Measurements of BHBA (mmol/L) in the blood samples with BHB-check plus, FSP-Neo and photometrical evaluation. Diff.=difference.

Cow ID	BHB-check (mmol/L)	FSP-Neo (mmol/L)	Diff. BHB-check & FSP-Neo (mmol/L)	Photometrical evaluation (mmol/L)	Diff. BHB-check & external lab. (mmol/L)	Diff. FSP-Neo & external lab. (mmol/L)
1	0.6	0.4	0.2	0.501[a]	0.10	-0.10
2	0.8	0.6	0.2	0.540[c]	0.26	0.06
3	0.6	0.4	0.2	0.513[c]	0.09	-0.11
4	0.5	0.3	0.2	0.468[c]	0.03	-0.17
5	0.6	0.4	0.2	0.398[b]	0.20	0.00
6	1.2	0.8	0.4	0.725[b]	0.48	0.08
Mean	0.717	0.483	0.233	0.524	0.193	-0.04

a: Hemolysis index: ++++, b: Hemolysis index: + c: Hemolysis index negative

B-2. Raw plot of 19 measurements

Figure A. Distribution of the 19 measurements of BHBA concentration (mmol/L) with BHB-check plus and Precision Neo. Orange line= threshold of 100% agreement.



B-3. Calculation of the correction factor

Table D. Calculation of the correction factor.

Cow	BHB-check (method 1)	Precision Neo (method 2)	(Method 1-Method 2)/method 2(gold)
1	1,50	0,90	0,667
2	1,40	0,60	1,333
3	1,80	1,00	0,800
4	1,40	0,70	1,000
5	1,90	0,90	1,111
6	1,70	0,80	1,125
7	1,80	1,30	0,385
8	1,40	0,80	0,750
9	1,00	0,40	1,500
10	1,90	1,20	0,583
11	1,00	0,50	1,000
12	1,80	1,00	0,800
13	1,80	0,80	1,250
14	1,40	0,80	0,750
15	1,80	0,70	1,571
16	2,10	1,30	0,615
17	2,00	0,70	1,857
18	0,90	0,60	0,500
19	2,20	1,20	0,833
20	0,6	0,4	0,500
21	0,8	0,6	0,333
22	0,6	0,4	0,500
23	0,5	0,3	0,667
24	0,6	0,4	0,500
25	1,2	0,8	0,500
Mean			**0,857**

B-4. Corrected data set

Table E. The dataset with corrected values of the BHB-check (D1). Yellow row = shift to D2 device. NA= empty cell.

Herd id	Cow id	Parity	Test 1	Test 2	Test 3	Test 4 corrected	Error code	Date	Parity group
1	1	parity_4	12	2	2	0,8	NA	16/09/2019	Older
1	3	parity_1	10	1	1	0,6	NA	16/09/2019	First
2	5	parity_2	12	1	1	0,6	NA	16/09/2019	Second
2	7	parity_4	10	1	1	0,4	NA	16/09/2019	Older
2	8	parity_1	10	1	1	0,4	NA	16/09/2019	First
2	9	parity_1	10	1	1	0,5	NA	16/09/2019	First

2	10	parity_3	10	1	1	0,7	NA	16/09/2019	Older
3	11	parity_5	10	1	1	0,6	NA	16/09/2019	Older
3	12	parity_3	12	4	4	1,2	NA	16/09/2019	Older
3	13	parity_2	10	1	1	0,5	NA	16/09/2019	Second
3	14	parity_2	10	1	1	0,7	NA	16/09/2019	Second
3	15	parity_1	10	1	1	0,5	NA	16/09/2019	First
4	16	parity_3	13	2	2	0,7	NA	17/09/2019	Older
4	17	parity_1	12	4	4	0,9	NA	17/09/2019	First
4	19	parity_3	10	2	2	1,0	NA	17/09/2019	Older
4	21	parity_2	10	1	1	0,5	NA	17/09/2019	Second
4	22	parity_8	10	1	1	0,8	NA	17/09/2019	Older
5	23	parity_3	10	1	1	0,9	NA	17/09/2019	Older
5	24	parity_3	10	1	1	0,5	NA	17/09/2019	Older
5	25	parity_2	10	1	1	0,5	NA	17/09/2019	Second
5	26	parity_5	10	2	1	1,0	NA	17/09/2019	Older
5	27	parity_4	10	1	1	1,0	NA	17/09/2019	Older
6	28	parity_4	12	1	1	0,7	NA	18/09/2019	Older
6	30	parity_4	10	2	1	0,5	NA	18/09/2019	Older
7	31	parity_3	10	1	1	0,4	NA	18/09/2019	Older
7	33	parity_3	10	2	2	0,8	NA	18/09/2019	Older
7	35	parity_1	10	1	1	0,6	NA	18/09/2019	First
7	36	parity_2	10	1	1	0,4	NA	18/09/2019	Second
7	37	parity_3	10	1	1	0,4	NA	18/09/2019	Older
8	40	parity_1	10	1	1	0,4	NA	18/09/2019	First
8	41	parity_1	10	1	1	0,3	NA	18/09/2019	First
8	42	parity_2	12	1	2	0,8	NA	18/09/2019	Second
8	43	parity_1	10	1	1	0,4	NA	18/09/2019	First
8	44	parity_3	12	3	3	1,1	NA	18/09/2019	Older
8	45	parity_2	10	1	1	0,9	NA	18/09/2019	Second
8	46	parity_2	10	1	1	0,4	NA	18/09/2019	Second
9	47	parity_3	10	1	1	0,9	NA	19/09/2019	Older
9	48	parity_3	10	1	1	0,6	NA	19/09/2019	Older
9	49	parity_2	10	1	1	0,8	NA	19/09/2019	Second
9	50	parity_3	10	1	1	0,8	NA	19/09/2019	Older
9	51	parity_2	10	2	2	0,7	NA	19/09/2019	Second
9	52	parity_2	10	1	1	0,9	NA	19/09/2019	Second
9	54	parity_2	12	1	1	0,6	NA	19/09/2019	Second
2	55	parity_1	10	1	1	0,6	NA	23/09/2019	First
2	56	parity_3	10	1	1	0,5	NA	23/09/2019	Older
2	57	parity_2	10	1	1	0,5	NA	23/09/2019	Second
2	58	parity_2	10	1	1	0,6	NA	23/09/2019	Second

1	60	parity_1	13	1	1	0,6	NA	23/09/2019	First
1	62	parity_1	10	1	1	0,4	NA	23/09/2019	First
1	64	parity_2	10	1	1	0,6	NA	23/09/2019	Second
1	66	parity_2	10	1	1	0,6	NA	23/09/2019	Second
1	67	parity_2	12	2	2	0,7	NA	23/09/2019	Second
4	69	parity_1	10	1	1	0,4	NA	24/09/2019	First
4	70	parity_1	13	1	1	0,7	NA	24/09/2019	First
4	71	parity_3	12	4	4	1,4	NA	24/09/2019	Older
4	72	parity_1	10	1	1	0,7	NA	24/09/2019	First
4	73	parity_3	10	2	2	0,9	NA	24/09/2019	Older
4	74	parity_3	10	1	1	0,4	NA	24/09/2019	Older
4	75	parity_1	10	1	1	0,6	NA	24/09/2019	First
5	77	parity_3	10	1	1	0,7	NA	24/09/2019	Older
5	78	parity_1	12	2	1	0,6	NA	24/09/2019	First
10	79	parity_1	10	1	1	0,4	NA	25/09/2019	First
10	80	parity_3	12	1	1	0,5	NA	25/09/2019	Older
10	81	parity_2	10	1	1	0,4	NA	25/09/2019	Second
10	82	parity_1	10	1	1	0,6	NA	25/09/2019	First
10	84	parity_4	10	1	1	0,4	NA	25/09/2019	Older
10	86	parity_2	12	4	4	1,0	NA	25/09/2019	Second
10	88	parity_2	10	1	1	0,5	NA	25/09/2019	Second
10	90	parity_1	10	1	1	0,4	NA	25/09/2019	First
10	93	parity_1	10	1	1	0,2	NA	25/09/2019	First
10	94	parity_1	10	1	1	0,5	NA	25/09/2019	First
10	95	parity_2	10	1	1	0,8	NA	25/09/2019	Second
10	96	parity_2	10	2	2	0,6	NA	25/09/2019	Second
11	97	parity_3	10	1	1	0,6	NA	26/09/2019	Older
11	98	parity_4	12	1	1	0,6	NA	26/09/2019	Older
11	99	parity_3	12	5	5	2,3	NA	26/09/2019	Older
11	100	parity_3	10	1	1	0,3	NA	26/09/2019	Older
11	101	parity_2	10	2	1	0,4	NA	26/09/2019	Second
11	102	parity_2	10	1	1	0,3	NA	26/09/2019	Second
11	103	parity_1	10	1	1	0,3	NA	26/09/2019	First
11	104	parity_1	10	1	1	0,6	NA	26/09/2019	First
11	105	parity_1	10	1	1	0,3	NA	26/09/2019	First
11	106	parity_1	10	1	1	0,3	NA	26/09/2019	First
11	107	parity_1	10	1	1	0,5	NA	26/09/2019	First
9	110	parity_5	14	1	1	0,8	NA	26/09/2019	Older
9	115	parity_2	10	1	2	0,5	NA	26/09/2019	Second
2	116	parity_1	10	1	1	0,5	NA	30/09/2019	First
2	117	parity_4	12	1	1	0,5	NA	30/09/2019	Older

2	118	parity_1	10	1	1	0,5	NA	30/09/2019	First
2	119	parity_4	10	1	1	0,8	NA	30/09/2019	Older
2	120	parity_2	10	1	1	0,8	NA	30/09/2019	Second
1	122	parity_3	10	1	1	0,4	NA	30/09/2019	Older
1	124	parity_3	10	1	1	0,4	NA	30/09/2019	Older
3	127	parity_4	12	2	2	1,0	NA	30/09/2019	Older
3	128	parity_2	12	1	1	0,8	NA	30/09/2019	Second
3	129	parity_2	12	1	1	0,6	NA	30/09/2019	Second
3	130	parity_2	10	3	2	0,9	NA	30/09/2019	Second
3	131	parity_2	10	1	1	0,8	NA	30/09/2019	Second
3	132	parity_1	10	1	1	0,6	NA	30/09/2019	First
4	133	parity_3	10	1	1	0,4	NA	01/10/2019	Older
4	134	parity_4	12	2	1	1,1	NA	01/10/2019	Older
4	136	parity_2	10	2	2	0,9	NA	01/10/2019	Second
4	137	parity_2	10	1	1	0,8	NA	01/10/2019	Second
4	139	parity_2	10	2	2	1,2	NA	01/10/2019	Second
4	140	parity_2	10	1	1	0,9	NA	01/10/2019	Second
4	141	parity_2	10	1	1	0,5	NA	01/10/2019	Second
4	142	parity_1	10	1	1	0,8	NA	01/10/2019	First
4	143	parity_3	10	2	2	1,1	NA	01/10/2019	Older
4	144	parity_1	10	1	1	0,5	NA	01/10/2019	First
4	145	parity_1	10	1	1	0,7	NA	01/10/2019	First
4	146	parity_1	10	1	1	0,5	NA	01/10/2019	First
4	147	parity_1	10	1	1	0,6	NA	01/10/2019	First
4	148	parity_1	10	1	1	1,0	NA	01/10/2019	First
12	151	parity_3	10	1	1	0,7	NA	01/10/2019	Older
12	152	parity_1	10	1	2	0,6	NA	01/10/2019	First
12	153	parity_2	12	1	1	0,6	NA	01/10/2019	Second
12	154	parity_4	13	1	1	0,5	NA	01/10/2019	Older
12	155	parity_3	11	1	2	0,5	NA	01/10/2019	Older
12	158	parity_4	10	1	1	0,9	NA	01/10/2019	Older
12	159	parity_4	10	1	1	0,8	NA	01/10/2019	Older
12	160	parity_4	10	1	1	0,9	NA	01/10/2019	Older
7	161	parity_5	10	1	1	0,6	NA	02/10/2019	Older
7	162	parity_5	10	1	1	0,9	NA	02/10/2019	Older
7	164	parity_5	10	1	1	0,9	NA	02/10/2019	Older
7	166	parity_3	10	1	1	0,5	NA	02/10/2019	Older
7	167	parity_2	10	1	1	0,5	NA	02/10/2019	Second
7	168	parity_2	10	1	1	0,8	NA	02/10/2019	Second
11	169	parity_1	12	2	1	0,4	NA	03/10/2019	First
11	170	parity_3	10	1	1	0,4	NA	03/10/2019	Older

11	172	parity_1	10	1	1	0,4	NA	03/10/2019	First
11	173	parity_4	10	1	1	0,6	NA	03/10/2019	Older
11	174	parity_3	10	1	1	0,7	NA	03/10/2019	Older
11	175	parity_3	10	1	1	0,3	NA	03/10/2019	Older
11	176	parity_3	12	4	3	1,2	NA	03/10/2019	Older
11	177	parity_2	10	1	1	0,5	NA	03/10/2019	Second
13	179	parity_4	10	1	1	0,5	NA	04/10/2019	Older
13	180	parity_4	10	1	1	0,7	NA	04/10/2019	Older
13	181	parity_4	10	1	1	0,5	NA	04/10/2019	Older
13	182	parity_1	10	2	1	0,6	NA	04/10/2019	First
13	183	parity_1	10	1	1	0,5	NA	04/10/2019	First
1	185	parity_1	10	1	1	0,9	NA	07/10/2019	First
2	188	parity_1	13	1	1	0,5	NA	07/10/2019	First
2	189	parity_2	10	1	1	0,8	NA	07/10/2019	Second
2	190	parity_5	10	1	1	0,8	NA	07/10/2019	Older
4	191	parity_5	10	1	1	0,7	NA	08/10/2019	Older
4	192	parity_4	11	2	2	0,6	NA	08/10/2019	Older
4	193	parity_4	10	1	1	0,9	NA	08/10/2019	Older
4	194	parity_4	11	2	2	1,2	NA	08/10/2019	Older
4	195	parity_4	10	1	1	0,9	NA	08/10/2019	Older
4	196	parity_3	10	2	2	0,8	NA	08/10/2019	Older
4	197	parity_3	10	1	1	0,8	NA	08/10/2019	Older
4	198	parity_2	10	1	1	0,6	NA	08/10/2019	Second
4	199	parity_1	10	1	1	0,6	NA	08/10/2019	First
4	200	parity_1	10	1	1	0,9	NA	08/10/2019	First
4	201	parity_2	13	1	1	0,8	NA	08/10/2019	Second
4	202	parity_2	10	1	1	0,3	NA	08/10/2019	Second
4	203	parity_2	11	3	3	1,2	NA	08/10/2019	Second
4	204	parity_2	10	1	1	0,7	NA	08/10/2019	Second
4	205	parity_2	10	1	1	0,8	NA	08/10/2019	Second
4	206	parity_2	10	1	1	0,8	NA	08/10/2019	Second
4	207	parity_2	10	1	1	0,6	NA	08/10/2019	Second
4	208	parity_2	10	1	1	0,8	NA	08/10/2019	Second
4	209	parity_1	10	1	1	0,6	NA	08/10/2019	First
4	210	parity_1	10	1	1	0,5	NA	08/10/2019	First
10	211	parity_3	10	1	1	0,4	NA	09/10/2019	Older
10	212	parity_2	10	1	1	0,6	NA	09/10/2019	Second
10	213	parity_1	10	1	1	0,4	NA	09/10/2019	First
10	214	parity_1	10	1	1	0,4	NA	09/10/2019	First
10	215	parity_4	10	2	2	0,8	NA	09/10/2019	Older
10	216	parity_1	10	1	1	0,6	NA	09/10/2019	First

10	219	parity_5	10	1	1	0,4	NA	09/10/2019	Older
7	220	parity_4	10	1	2	0,6	NA	09/10/2019	Older
7	221	parity_3	10	1	1	0,4	NA	09/10/2019	Older
7	222	parity_3	10	2	2	0,8	NA	09/10/2019	Older
7	223	parity_2	10	1	1	0,7	NA	09/10/2019	Second
7	224	parity_1	10	1	1	0,4	NA	09/10/2019	First
7	225	parity_1	10	1	1	0,4	NA	09/10/2019	First
7	226	parity_1	10	1	1	0,4	NA	09/10/2019	First
7	227	parity_1	10	1	1	0,5	NA	09/10/2019	First
11	228	parity_3	10	1	1	1,0	NA	10/10/2019	Older
11	229	parity_7	10	1	1	0,2	NA	10/10/2019	Older
11	230	parity_3	10	1	1	0,8	NA	10/10/2019	Older
11	231	parity_3	10	1	1	0,6	NA	10/10/2019	Older
11	232	parity_3	10	1	1	0,8	NA	10/10/2019	Older
11	233	parity_3	11	2	1	0,9	NA	10/10/2019	Older
11	234	parity_2	10	1	1	1,0	NA	10/10/2019	Second
11	235	parity_2	11	1	1	0,9	NA	10/10/2019	Second
11	236	parity_2	10	1	1	0,6	NA	10/10/2019	Second
11	238	parity_1	12	1	1	0,6	NA	10/10/2019	First
11	239	parity_1	10	1	1	0,5	NA	10/10/2019	First
11	240	parity_1	10	1	1	0,6	NA	10/10/2019	First
11	241	parity_1	10	1	1	0,5	NA	10/10/2019	First
11	242	parity_1	10	1	1	0,8	NA	10/10/2019	First
11	243	parity_1	10	2	1	0,4	NA	10/10/2019	First
13	244	parity_4	10	1	1	0,6	NA	11/10/2019	Older
13	245	parity_3	10	1	1	0,6	NA	11/10/2019	Older
13	247	parity_1	10	1	1	0,6	NA	11/10/2019	First
13	248	parity_1	10	1	1	0,4	NA	11/10/2019	First
1	249	parity_2	10	1	1	0,6	NA	14/10/2019	Second
1	251	parity_1	10	1	1	0,6	NA	14/10/2019	First
1	254	parity_1	10	1	1	0,5	NA	14/10/2019	First
2	256	parity_1	10	1	1	0,6	NA	14/10/2019	First
2	258	parity_1	10	1	1	0,5	NA	14/10/2019	First
2	259	parity_1	10	1	1	0,2	NA	14/10/2019	First
2	261	parity_1	10	1	1	0,7	NA	14/10/2019	First
3	263	parity_1	10	1	1	0,4	NA	14/10/2019	First
3	265	parity_4	12	3	3	1,3	NA	14/10/2019	Older
3	267	parity_1	10	1	2	0,5	NA	14/10/2019	First
4	269	parity_3	10	1	2	0,5	NA	15/10/2019	Older
4	270	parity_3	10	2	1	1,1	NA	15/10/2019	Older
4	271	parity_3	10	2	2	1,0	NA	15/10/2019	Older

4	273	parity_2	10	1	1	0,6	NA	15/10/2019	Second
4	274	parity_2	10	2	2	0,7	NA	15/10/2019	Second
4	275	parity_2	10	1	1	1,0	NA	15/10/2019	Second
4	276	parity_2	10	1	1	0,9	NA	15/10/2019	Second
4	277	parity_2	10	1	1	0,6	NA	15/10/2019	Second
4	278	parity_2	10	3	3	0,9	NA	15/10/2019	Second
4	280	parity_1	10	1	1	0,6	NA	15/10/2019	First
12	281	parity_1	10	1	1	0,4	NA	15/10/2019	First
12	282	parity_5	10	2	2	0,8	NA	15/10/2019	Older
12	284	parity_3	10	2	2	0,6	NA	15/10/2019	Older
12	285	parity_3	11	1	1	0,6	NA	15/10/2019	Older
12	286	parity_6	10	1	1	0,7	NA	15/10/2019	Older
12	287	parity_1	10	1	1	0,4	NA	15/10/2019	First
12	288	parity_5	10	2	2	0,6	NA	15/10/2019	Older
12	289	parity_2	12	4	4	1,1	NA	15/10/2019	Second
12	291	parity_3	10	1	1	0,6	NA	15/10/2019	Older
1	292	parity_5	12	3	3	1,4	NA	21/10/2019	Older
1	294	parity_5	10	2	2	1,0	NA	21/10/2019	Older
1	295	parity_3	11	2	2	0,9	NA	21/10/2019	Older
2	296	parity_2	10	2	2	0,6	NA	21/10/2019	Second
2	297	parity_2	10	2	2	0,9	NA	21/10/2019	Second
2	299	parity_4	10	1	1	0,7	NA	21/10/2019	Older
2	300	parity_2	10	2	2	1,1	NA	21/10/2019	Second
14	302	parity_3	10	1	1	0,5	NA	22/10/2019	Older
14	303	parity_3	10	1	1	0,3	NA	22/10/2019	Older
14	304	parity_3	10	1	1	0,4	NA	22/10/2019	Older
14	306	parity_3	10	1	1	0,5	NA	22/10/2019	Older
14	307	parity_1	12	1	1	0,5	NA	22/10/2019	First
14	308	parity_1	11	1	1	0,5	NA	22/10/2019	First
10	310	parity_2	10	1	1	0,3	NA	23/10/2019	Second
10	311	parity_2	11	1	1	0,4	NA	23/10/2019	Second
10	312	parity_1	11	1	1	0,3	NA	23/10/2019	First
10	313	parity_1	10	1	1	0,4	NA	23/10/2019	First
10	315	parity_1	10	1	1	0,5	NA	23/10/2019	First
10	316	parity_1	10	2	1	0,4	NA	23/10/2019	First
10	319	parity_1	10	1	1	0,4	NA	23/10/2019	First
10	320	parity_1	12	1	1	0,5	NA	23/10/2019	First
9	322	parity_1	10	1	1	0,4	NA	24/10/2019	First
9	324	parity_1	10	1	1	0,3	NA	24/10/2019	First
9	325	parity_1	10	1	1	0,4	NA	24/10/2019	First
9	327	parity_2	10	1	1	0,8	NA	24/10/2019	Second

11	328	parity_1	10	2	2	0,9	NA	24/10/2019	First
11	330	parity_1	10	1	1	0,4	NA	24/10/2019	First
11	333	parity_4	10	1	1	0,8	NA	24/10/2019	Older
11	335	parity_3	10	1	1	0,7	NA	24/10/2019	Older
11	336	parity_3	10	1	1	0,7	NA	24/10/2019	Older
11	337	parity_2	11	2	2	0,9	NA	24/10/2019	Second
11	338	parity_2	12	2	2	1,0	NA	24/10/2019	Second
11	339	parity_1	10	1	1	0,6	NA	24/10/2019	First
11	341	parity_1	10	1	1	0,6	NA	24/10/2019	First
11	342	parity_1	10	1	1	0,8	NA	24/10/2019	First
7	344	parity_3	10	1	1	0,4	NA	24/10/2019	Older
7	345	parity_3	10	3	3	0,8	NA	24/10/2019	Older
7	346	parity_2	10	1	1	0,6	NA	24/10/2019	Second
7	347	parity_4	12	2	2	1,0	NA	24/10/2019	Older
7	348	parity_2	10	2	2	0,9	NA	24/10/2019	Second
4	349	parity_2	10	1	1	0,8	NA	28/10/2019	Second
4	350	parity_2	10	1	1	0,8	NA	28/10/2019	Second
4	351	parity_2	10	1	1	1,0	NA	28/10/2019	Second
4	352	parity_2	10	1	1	0,8	NA	28/10/2019	Second
4	353	parity_2	10	1	1	1,0	NA	28/10/2019	Second
4	354	parity_2	10	1	1	0,9	NA	28/10/2019	Second
4	355	parity_1	10	2	2	1,0	NA	28/10/2019	First
4	356	parity_1	10	1	1	0,8	NA	28/10/2019	First
4	357	parity_1	12	1	1	0,5	NA	28/10/2019	First
4	358	parity_1	10	1	1	1,0	NA	28/10/2019	First
4	360	parity_3	10	2	2	1,0	NA	28/10/2019	Older
4	361	parity_3	10	2	2	1,0	NA	28/10/2019	Older
4	362	parity_3	10	1	1	0,8	NA	28/10/2019	Older
4	363	parity_3	10	1	1	1,0	NA	28/10/2019	Older
4	364	parity_3	10	3	3	1,1	NA	28/10/2019	Older
4	365	parity_3	10	1	1	1,1	NA	28/10/2019	Older
4	367	parity_2	10	1	1	1,2	NA	28/10/2019	Second
4	368	parity_2	10	1	1	0,6	NA	28/10/2019	Second
14	371	parity_2	10	1	1	0,7	NA	29/10/2019	Second
3	372	parity_1	12	1	1	0,6	NA	30/10/2019	First
3	373	parity_4	12	3	3	1,7	NA	30/10/2019	Older
3	376	parity_1	10	1	1	0,6	NA	30/10/2019	First
3	379	parity_1	10	1	1	0,8	NA	30/10/2019	First
3	380	parity_3	10	1	1	1,0	NA	30/10/2019	Older
10	385	parity_2	10	1	1	0,5	NA	30/10/2019	Second
7	387	parity_5	10	2	2	1,1	NA	30/10/2019	Older

7	388	parity_5	10	1	1	0,8	NA	30/10/2019	Older
7	389	parity_2	10	2	1	0,8	NA	30/10/2019	Second
7	390	parity_1	10	2	2	0,9	NA	30/10/2019	First
7	391	parity_1	10	1	1	0,7	NA	30/10/2019	First
11	392	parity_1	10	1	1	0,9	NA	31/10/2019	First
11	393	parity_1	12	1	1	1,2	NA	31/10/2019	First
11	394	parity_1	10	1	1	1,4	NA	31/10/2019	First
11	395	parity_1	10	2	1	1,0	NA	31/10/2019	First
11	396	parity_1	10	1	1	1,1	NA	31/10/2019	First
11	397	parity_4	13	1	1	1,1	NA	31/10/2019	Older
11	399	parity_1	10	1	1	1,2	NA	31/10/2019	First
11	400	parity_1	10	1	1	1,5	NA	31/10/2019	First
11	401	parity_3	10	1	1	0,9	NA	31/10/2019	Older
11	402	parity_3	12	2	2	1,9	NA	31/10/2019	Older
11	403	parity_3	10	1	1	1,4	NA	31/10/2019	Older
9	406	parity_6	10	1	1	1,5	NA	31/10/2019	Older
2	411	parity_2	10	1	1	1,3	NA	04/11/2019	Second
1	413	parity_1	10	1	1	0,8	NA	04/11/2019	First
1	414	parity_3	10	2	2	1,4	NA	04/11/2019	Older
1	416	parity_3	10	1	1	1,8	NA	04/11/2019	Older
1	418	parity_2	12	2	2	1,8	NA	04/11/2019	Second
4	420	parity_7	11	2	1	0,9	NA	05/11/2019	Older
4	421	parity_2	10	1	1	0,7	NA	05/11/2019	Second
4	422	parity_3	10	1	1	0,8	NA	05/11/2019	Older
4	423	parity_4	10	1	1	1,2	NA	05/11/2019	Older
4	424	parity_3	10	1	1	0,7	NA	05/11/2019	Older
4	426	parity_2	10	2	2	1,3	NA	05/11/2019	Second
4	427	parity_3	10	2	2	1,4	NA	05/11/2019	Older
4	428	parity_3	10	1	1	0,9	NA	05/11/2019	Older
4	429	parity_2	10	1	1	1,5	NA	05/11/2019	Second
4	430	parity_2	10	1	1	1,1	NA	05/11/2019	Second
4	431	parity_3	10	2	2	1,6	NA	05/11/2019	Older
7	433	parity_4	10	1	1	1,7	NA	06/11/2019	Older
7	434	parity_3	11	2	2	2,2	NA	06/11/2019	Older
7	435	parity_2	12	1	1	1,9	NA	06/11/2019	Second
7	438	parity_2	10	1	1	1,3	NA	06/11/2019	Second
7	439	parity_5	14	1	1	0,9	NA	06/11/2019	Older
13	441	parity_4	10	1	1	1,2	NA	07/11/2019	Older
13	442	parity_3	10	2	2	1,3	NA	07/11/2019	Older
13	444	parity_6	10	1	1	0,8	NA	07/11/2019	Older
13	445	parity_1	10	1	1	0,6	NA	07/11/2019	First

13	446	parity_1	10	1	1	0,7	NA	07/11/2019	First
11	447	parity_6	10	1	1	1,1	NA	07/11/2019	Older
11	448	parity_3	10	1	1	0,5	NA	07/11/2019	Older
11	450	parity_2	10	1	1	0,7	NA	07/11/2019	Second
11	451	parity_2	10	1	1	0,5	NA	07/11/2019	Second
11	452	parity_2	10	1	1	0,6	NA	07/11/2019	Second
11	453	parity_2	10	1	1	0,5	NA	07/11/2019	Second
11	454	parity_2	10	2	2	0,8	NA	07/11/2019	Second
2	458	parity_6	10	1	1	0,8	NA	11/11/2019	Older
2	461	parity_4	10	1	1	0,9	NA	11/11/2019	Older
2	462	parity_1	10	2	2	0,9	NA	11/11/2019	First
1	463	parity_6	10	1	1	1,0	NA	11/11/2019	Older
1	465	parity_3	10	1	1	1,0	NA	11/11/2019	Older
3	469	parity_4	10	1	1	0,9	NA	11/11/2019	Older
3	470	parity_3	10	1	1	1,1	NA	11/11/2019	Older
3	471	parity_3	10	1	1	0,8	NA	11/11/2019	Older
4	474	parity_2	10	2	2	1,0	NA	12/11/2019	Second
4	475	parity_1	12	2	2	1,3	NA	12/11/2019	First
4	476	parity_3	10	2	2	1,3	NA	12/11/2019	Older
4	478	parity_3	10	3	3	1,6	NA	12/11/2019	Older
4	480	parity_1	12	1	1	0,8	NA	12/11/2019	First
4	481	parity_6	13	1	1	0,9	NA	12/11/2019	Older
11	482	parity_5	10	1	1	1,1	NA	14/11/2019	Older
11	483	parity_5	10	1	1	0,6	NA	14/11/2019	Older
11	484	parity_3	10	1	1	0,6	NA	14/11/2019	Older
11	486	parity_2	10	1	1	0,7	NA	14/11/2019	Second
11	487	parity_2	10	1	1	0,6	NA	14/11/2019	Second
11	488	parity_1	10	1	1	0,4	NA	14/11/2019	First

Appendix C – Herd level prevalence

Table F. Herd prevalence Tmilk

Test 1	
Herd	**Prevalence**
1	0.2272727
2	0.1034483
3	0.3181818
4	0.1098901
5	0.1428571
6	0.5
7	0.08823529
8	0.2857143
9	0.1428571
10	0.1071429
11	0.1323529
12	0.1764706
13	0
14	0.1428571

Table G. Herd prevalence Uman

Test 2	
Herd	**Prevalence**
1	0.04545455
2	0
3	0.1818182
4	0.06593407
5	0
6	0
7	0.02941176
8	0.1428571
9	0
10	0.03571429
11	0.02941176
12	0.05882353
13	0
14	0

Table H. Herd prevalence Ucat

Test 3	
Herd	**Prevalence**
1	0.04545455
2	0
3	0.1363636
4	0.06593407
5	0
6	0
7	0.02941176
8	0.1428571
9	0
10	0.03571429
11	0.02941176
12	0.05882353
13	0
14	0

Table I. Herd prevalence Blorig

Test 4	
Herd	**Prevalence**
1	0.5454545
2	0.3793103
3	0.5454545
4	0.6813187
5	0.5714286
6	0.5
7	0.5588235
8	0.4285714
9	0.6428571
10	0.1785714
11	0.4117647
12	0.7058824
13	0.2857143
14	0.1428571

Table J. Herd prevalence Blcorr-

Test 4 corr.	
Herd	**Prevalence**
1	0.1818182
2	0.03448276
3	0.1363636
4	0.1428571
5	0
6	0
7	0.1176471
8	0
9	0.07142857
10	0
11	0.1176471
12	0
13	0.1428571
14	0

Appendix D – Study 2

D-1. Raw data

Table K. Raw data from study 2. White felts= D1 measurements, Orange felt=D2 measurements. Green felt= cow with milk fever C=Calving, M=missed measurements, !!! expected calving would have replaced the seventh measurement.

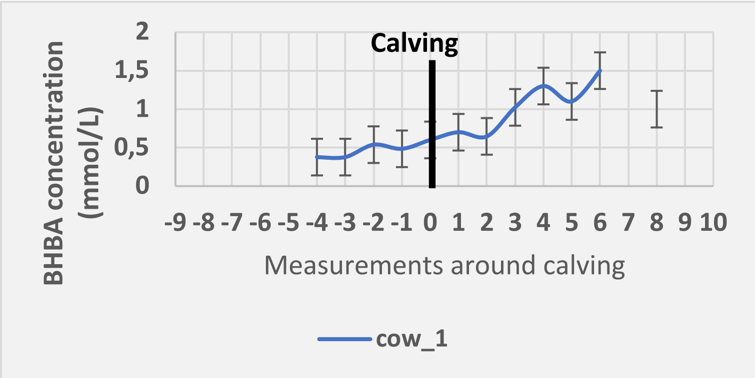
Non-corr.	Measurements (blood BHBA mmol/L)												
Cows	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
Cow_1	0.7	0.7	1	0.9	C	1.3	1.2	1.9	1.3	1.1	1.5	M	1
Cow_2	0.8	1.2	C	2.4	1.3	1.7	1.5	1.0	0.6	1.3	0.8	0.7	0.8
Cow_3	0.5	0.7	0.9	1	C	0.8	1.1	0.8	1.8	0.9	0.9	1.1	0.6
Cow_4	0.7	0.5	0.8	0.6	1.1	0.9	C	1.5	0.8	0.9	M	0.8	1.4
Cow_5	0.7	1	1	1	1.4	1.2	1	2	0.8	C	1	0.8	1.1
Cow_6	1	0.9	0.4	C	1.1	0.9	0.9	0.4	0.8	M	0.5	0.4	0.7
Cow_7	1.7	1.2	0.8	0.9	1.6	C	3	1.1	2	1.6	1.3	1	1.4
Cow_8	1	0.9	1.7	0.6	1.0	1	M	0.8	C	1.1	1.2	0.9	1.3
Cow_9	1.3	1.2	1	0.8	1.3	1	M	0.7	C	1.4	0.8	M	1.2
Cow_10	0.5	0.8	0.7	0.9	1.0	C	1.3	0.5	1.2	0.9	0.9	1.1	0.9

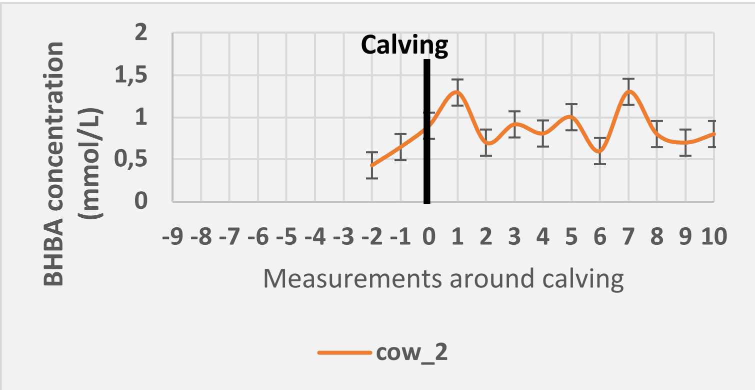
D-2. Corrected measurements of D1.

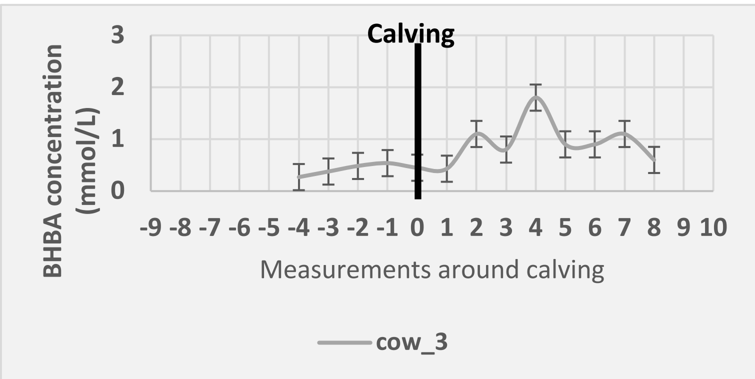
Table L.Corrected measurements of D1. White felts= D1 measurements, Orange felt=D2 measurements. Green felt= cow with milk fever, C=Calving, M=missed measurements, !!! expected calving would have replaced the seventh measurement.

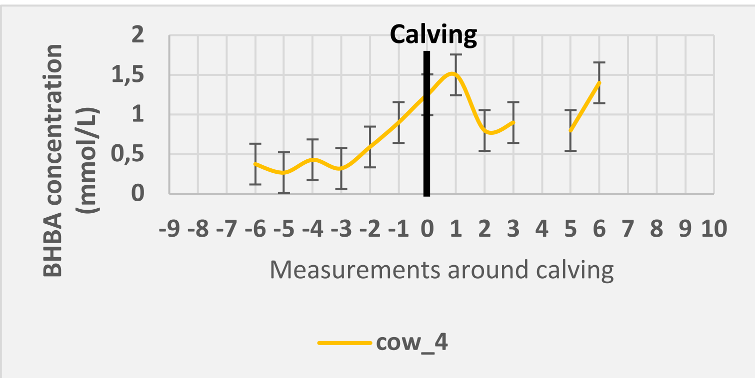
Corr.	Measurements (blood BHBA mmol/L)												
Cows	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
Cow_1	0.4	0.4	0.5	0.5	K	0.7	0.6	1.0	1.3	1.1	1.5	M	1
Cow_2	0.4	0.6	K	1.3	0.7	0.9	0.8	1.0	0.6	1.3	0.8	0.7	0.8
Cow_3	0.3	0.4	0.5	0.5	K	0.4	1.1	0.8	1.8	0.9	0.9	1.1	0.6
Cow_4	0.4	0.3	0.4	0.3	0.6	0.9	K	1.5	0.8	0.9	M	0.8	1.4
Cow_5	0.4	0.5	0.5	0.5	0.8	1.2	1	2	0.8	K	1	0.8	1.1
Cow_6	0.5	0.5	0.2	K	1.1	0.9	0.9	0.4	0.8	M	0.5	0.4	0.7
Cow_7	0.9	0.6	0.4	0.5	1.6	K	3	1.1	2	1.6	1.3	1	1.4
Cow_8	0.5	0.5	0.9	0.3	1	1	M	0.8	K	1.1	1.2	0.9	1.3
Cow_9	0.7	0.6	0.5	0.4	1.3	1	M	0.7	K	1.4	0.8	M	1.2
Cow_10	0.3	0.4	0.4	0.5	1	K	1.3	0.5	1.2	0.9	0.9	1.1	0.9

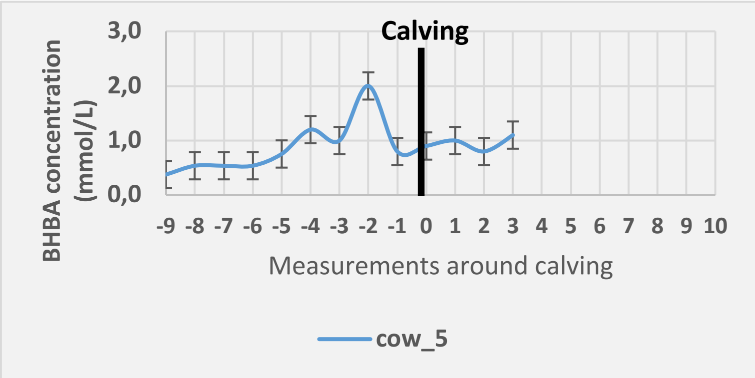
D-3. Variation of BHBA concentration in each cow using corrected D1

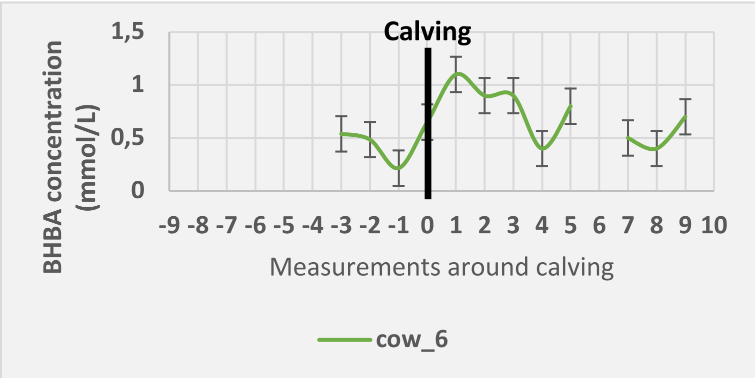


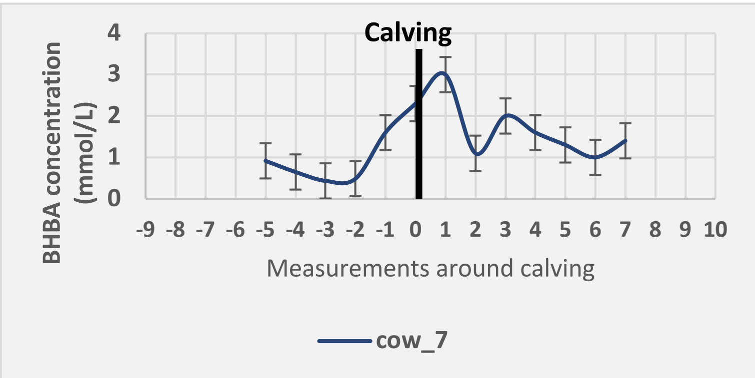


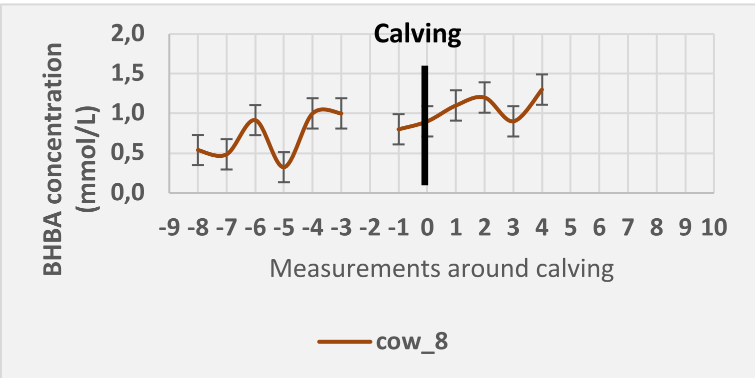


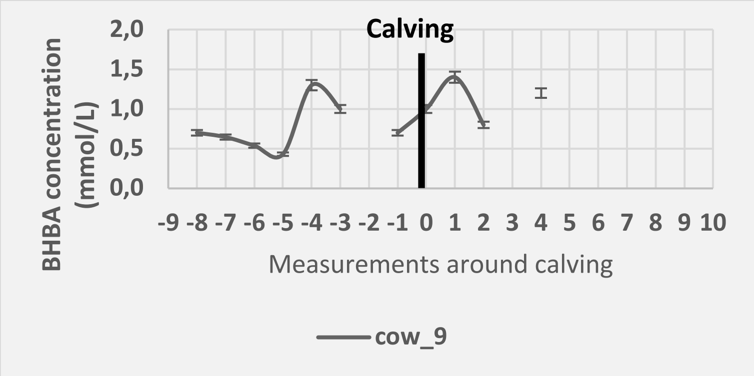


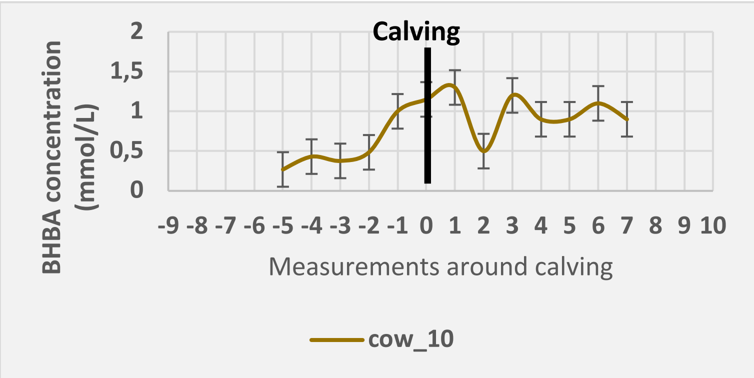

Calving
3,0
2,0
1,0
0,0
BHBA concentration (mmol/L)
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Measurements around calving
cow_5


Calving
1,5
1
0,5
0
BHBA concentration (mmol/L)
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Measurements around calving
cow_6


Calving
4
3
2
1
0
BHBA concentration (mmol/L)
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Measurements around calving
cow_7


Calving
2,0
1,5
1,0
0,5
0,0
BHBA concentration (mmol/L)
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Measurements around calving
cow_8





D-4. Proportion of SCK in the weeks postpartum

Table 13. Calculated proportion of SCK (≥1.2 mmol/L blood BHBA) in the measurements pp put together to four weeks with three weekly measurements.

	1 wk after			2 wk after			3 wk after			4 wk after
	first	second	third	first	second	third	first	second	third	first
	0,7	0,6	1,0	1,3	1,1	1,5	1,0			
	1,3	0,7	0,9	0,8	1,0	0,6	1,3	0,8	0,7	0,8
	0,4	1,1	0,8	1,8	0,9	0,9	1,1	0,6		
	1,5	0,8	0,9	0,8	1,4					
	1,0	0,8	1,1							
	1,1	0,9	0,9	0,4	0,8		0,5	0,4	0,7	
	3,0	1,1	2,0	1,6	1,3	1,0	1,4			
	1,1	1,2	0,9	1,3						
	1,4	0,8		1,2						
	1,3	0,5	1,2	0,9	0,9	1,1	0,9			
n	10	10	9	9	7	5	6	3	2	1
≥1,2 mmol/L	5	1	2	5	2	1	2	0	0	0
<1,2 mmol/L	5	9	7	4	5	4	4	3	2	1
Proportion	0,50	0,10	0,22	0,56	0,29	0,20	0,33	0	0	0

D-5. Relative risk

Table M. Amount of cows with above or below 0.7 mmol/L blood BHBA and whether or not they develop SCK pp (≥1.2 mmol/L blood BHBA).

Cow	≥7 mmol/L BHBA	<7 mmol/L BHBA	SCK+ (≥1.2 mmol/L pp)	SCK- (<1.2 mmol/L pp)
1		x	x	
2		x	x	
3		x	x	
4	x		x	
5	x			x
6		x		x
7	x		x	
8	x		x	
9	x		x	
10	x		x	
Total	**6**	**4**	**8**	**2**

Table N. Calculation of RR.

		SCK pp		
		+	-	Sum
cut-point	>7	5	1	6
(mmol/L blood BHBA prepartum)	<7	3	1	4
	Sum	8	2	10

Yates Corrected Chi-Square			0,234	
P-Value			0,628	
			95	% confidence limits
			Lower	Upper
Prevalence:		P	0,800	0,552	1,048
Relative Risk		RR	1,111	0,569	2,170
z-aplha	1,95996398				