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Master Thesis

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Assessment of calf health status within the first week in Icelandic dairy herds

A pilot study including clinical examinations and serum Brix% measurements

Supervisors: Nina Dam Otten, Lector and Dorte Bay Lastein, Associate professor

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Title and subtitle:	Assessment of calf health status within the first week in Icelandic dairy herds – A pilot study with clinical examinations and serum Brix% measurements.
Topic description:	The purpose of this project is to investigate the health of neonatal calves in Icelandic dairy farms. We will examine the literature for current evidence about the importance of general health in young calves in regard to having healthy future dairy cows. We will assess the health of 1 to 7 day old calves with clinical examinations and serum Brix% measurement, as well as evaluate calf and colostrum management on farms through a series of questions.
Supervisor:	Nina Dam Otten, Lector and Dorte Bay Lastein, Associate Professor
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Preface

This master's thesis is part of the Master of Science degree in Veterinary Medicine at the University of Copenhagen - Faculty of Health and Medical Sciences.

The purpose of the study is to research the health of neonatal Icelandic dairy calves, as well as elucidate the importance of farm management on the passive transfer of immunity.

We would like to thank our supervisors Nina Dam Otten and Dorte Bay Lastein for thorough guidance throughout the project.

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Annika Sámalsdóttir Olsen

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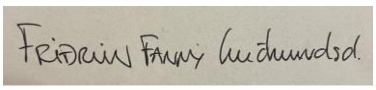

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Abstract

Calves are born essentially agammaglobulinemic and good colostrum management is therefore essential for calves in order to achieve passive transfer of immunity. Inadequate absorption of IgG from colostrum is known as failure of passive transfer and has been associated with an increased risk of morbidity and mortality. Although limited, previous data from Icelandic calves report lower levels of serum IgG than the recommended 10g/L and low levels in comparison to reports from calves of other breeds. This study aims to assess the health of neonatal Icelandic calves and evaluate the difference in Brix% among different calf-level and farm-management variables, as well as assess the risk of FPT within different colostrum management factors.

In total, 37 dairy farms in Iceland were visited, and interviews regarding colostrum management were conducted with all farmers. A total of 75 calves in the age of 1 to 7 days were included in the study. All calves were clinically assessed on 12 parameters and rectal temperature, and weight and heart-girth measurement were noted for 47 out of 75 calves. Serum Brix% was measured using digital Brix refractometers.

Results of this study show a prevalence of disease at 4%, of which the majority was caused by enteric disease. Mean weight and heart-girth were 32.9kg and 79.7cm, respectively, and the correlation between these variables was significant with $R = 0.6$ ($p < 0.01$). Mean and median Brix% were 7.8% and 7.9%, respectively, and the prevalence of FPT was 66.7%, when using 8.1% as the cut-off value. Mann-Whitney and Kruskal-Wallis models were used to determine significant differences in median Brix% among different calf-level and farm-management variables. There was only a significant difference ($p < 0.01$) in median Brix% for calves fed within 2 hours after birth and those fed 4-6 hours after birth. Fischer's exact model was used to determine significant differences in risk of FPT among farm-management groups, however, no significant differences were found in any of these groups.

To conclude, the serum Brix% levels of the calves were below the recommended levels and a majority of calves had FPT, despite of a low prevalence of disease. In general, there is a need for further research in the field of transfer of passive immunity in Icelandic dairy calves as well as there is room for improvement in the management of colostrum on Icelandic dairy farms.

Resumé

Kalve bliver født nærmest agammaglobulinæmiske og god kolostrum management er derfor en nødvendighed for at kalve kan opnå en tilstrækkelig grad af passiv immunisering. Utilstrækkelig absorption af IgG fra kolostrum bliver nævnt ”failure of passive transfer” og er blevet associeret med øget risiko for morbiditet og mortalitet. Om end der er få af dem, har tidligere studier vedrørende Islandske kalve vist et lavere niveau af serum IgG end de anbefalede 10g/L, samt et lavt niveau sammenlignet med studier med kalve af andre racer. Formålet med dette studie var at vurdere sundheden hos neonatale Islandske kalve og at evaluere forskellen i Brix% mellem forskellige variable på kalveniveau og indenfor besætningsmanagement, samt at vurdere risikoen for FPT blandt forskellige faktorer vedrørende kolostrummanagement.

Tilsammen 37 kvægbesætninger på Island blev besøgt og alle landmænd blev interviewet vedrørende kolostrummanagement. Tilsammen 75 kalve i alderen 1 til 7 dage var inkluderet i undersøgelsen. Alle kalve blev vurderet på 12 kliniske parametre samt rektal temperatur, og vægt og brystmål blev noteret fra 47 ud af 75 kalve. Serum Brix% blev målt ved brug af digitale Brix refraktometre.

Resultater fra studiet viser en sygdomsforekomst på 4%, hvor størstedelen var forårsaget af enterisk sygdom. Gennemsnitsvægt og -brystmål var hhv. 32.9kg og 79.7cm og der var en signifikant korrelation imellem dem med $R = 0.6$ ($p < 0.01$). Gennemsnit og median for Brix% var hhv. 7.8% og 7.9% og prævalensen af FPT var 66.7%, når grænseværdien på 8.1% blev brugt. Mann-Whitney og Kruskal-Wallis test blev anvendt til at påvise signifikante forskelle i median Brix% mellem forskellige variable på kalveniveau og indenfor besætningsmanagement. Der blev kun fundet signifikant forskel ($p < 0.01$) i median Brix% for kalve, der blev fodret indenfor 2 timer fra fødslen og dem, der blev fodret 4 til 6 timer efter fødslen. Fischer’s exact test blev anvendt til at påvise signifikante forskelle i risikoen for FPT blandt besætningsmanagement-grupper, der blev dog ikke fundet signifikante forskelle indenfor nogle disse grupper.

Konklusionen er, at kalvenes serum Brix% niveauer lå under det anbefalede niveau og at størstedelen af kalvene havde FPT, selvom sygdomsforekomsten var lav. Overordnet er der behov for yderligere undersøgelser vedrørende overførslen af passiv immunitet hos Islandske kalve udover, at der er plads til forbedring indenfor kolostrummanagement på Islandske malkekvægsbesætninger.

Keywords

Calf, Icelandic Cattle, IgG, Failure of Passive Transfer, FPT, Colostrum Managment

Abbreviations

IgG = Immunoglobulin G

FPT = Failure of passive transfer

APT = Adequate passive transfer

SD = Standard deviation

IQR = Interquartile range

RID = Radial immunodiffusion

RML = Icelandic agriculture centre (Ráðgjafamiðstöð landbúnaðarins)

1. Background

Calves, like other ruminants, are born essentially agammaglobulinemic. Because their placenta is of the epitheliochorial type, it does not allow passage of immunoglobulins, which leaves the neonate calf highly susceptible to infections and consumption of colostrum is therefore vital for the calf's health. When calves do not absorb enough immunoglobulin through colostrum, failure of passive transfer of immunity (FPT) can occur (Smith et al., 2020). FPT is defined as an IgG concentration below 10.0g/L in calves from 1 to 7 days of age (Tyler et al., 1996). On the other hand, when the IgG concentration is above 10.0 g/L, the calf is considered to have an adequate passive transfer of immunity (APT).

Both the individual calf and the dairy industry are affected by FPT in several different ways, e.g. multiple studies have reported associations between serum IgG concentration, and mortality and morbidity (Cuttance et al., 2018; Furman-Fratczak et al., 2011; Godden, 2008; Lora, Gottardo, et al., 2018). Because of the higher mortality and morbidity risks, FPT is a contributor to economic loss in the dairy industry (Raboisson et al., 2016). It remains unclear whether FPT affects the productivity and performance of the individual adult cow. However, a study reported a higher culling rate among cows, which had the lowest IgG concentrations shortly after birth, and 21.5% died or were culled because of low production (DeNise et al., 1989). Contrarily, a study from a pasture-based system did not report any significant effect of FPT on productivity, performance, or mortality in heifers over 12 months of age (Cuttance et al., 2019). Therefore, the effects of FPT on a long-term scale are yet to be uncovered.

In Iceland there is limited data available on FPT due to lack of research. The Icelandic dairy cow is highly genetically distinct from other Western European cattle and genetic research shows that the Icelandic cattle is most closely related to Northern, Eastern and Western Finn cattle and Swedish Mountain cattle (Gautason et al., 2020). Genetic differences, as well as the fact that the breed has been largely isolated for over 1000 years, might influence such factors as IgG and FPT (Gautason et al., 2020).

1.1 Risk Factors of Failure of Passive Transfer

FPT is, on its own, a risk factor for poor health, as calves with a lower concentration of immunoglobulins will be more at risk of infections (Furman-Fratczak et al., 2011). On the contrary, poor calf health can also be a risk factor for FPT. A calf, that experiences challenges in its first days of life e.g. in form of a difficult calving process, can have low vigour, which in turn can affect its ability to consume a sufficient volume of colostrum, depending on the feeding-method (Furman-Fratczak et al., 2011).

The most common health issues in neonatal calves include enteric- and respiratory infections (Agerholm et al., 1993; Gulliksen, Lie, Løken, et al., 2009; Svensson et al., 2006) and some of the major pathogens that have been isolated from calves with pneumonia include *Pasteurella multocida* and *Trueperella pyogenes*. Pathogens commonly isolated from calves with diarrhoea include Rotavirus and *Cryptosporidium parvum*. Additionally, types of *Escherichia coli* (*E. coli*) have been isolated from both calves with pneumonia and diarrhoea (Svensson et al., 2006). knowledge is limited, one source has stated, that the primary pathogen causing diarrhoea in young dairy calves in Iceland is *E. coli* (Harðarson, 2021). *Cryptosporidium* is commonly occurring, but it is unclear whether it causes illness in the calves, whilst *Salmonella* infections are rare (Harðarson, 2021). Pneumonia seems to be less widespread amongst calves in Iceland than in other western European countries, and according to Harðarson (2021) viruses affecting the respiratory system are not commonly reported in Icelandic dairy calves. However, poor environmental conditions can cause immunosuppression and facilitate the growth of opportunistic pathogens leading to bacterial pneumonia (Harðarson, 2021). A study by Furman-Fratczak et. al. (2011) observed that calves with an adequate transfer of passive immunity at 30 to 60 hours after birth, did not become ill of diarrhoea and pneumonia in the first two weeks of life. The calves also had lower morbidity and intensity of disease than calves with FPT. Calves with an IgG concentration > 15g/L did not contract respiratory tract infections in the same study (Furman-Fratczak et al., 2011).

In order to increase the general health of young calves, it is paramount to investigate what causes failure of passive transfer, and how the number of calves with acceptable IgG levels can be increased. The cause of FPT is multifactorial, but the on-farm colostrum management poses a major risk, and these risk factors include the colostrum quality, age at first feeding, and method and volume of the first colostrum feeding (Besser et al., 1991; Fischer et al., 2018; Lora, Barberio, et al., 2018; Morin et al., 1997; Renaud et al., 2020).

1.2 Colostrum Management

In their first days of life, calves require colostrum for the development of the immune system, as well as for energy. Colostrum contains several different components of nutrients including fat, lactose, and protein, which includes immunoglobulins (Ig). The main Ig component in colostrum is IgG_1, followed by IgG_2, IgM, and IgA. It has been internationally recommended, that the IgG concentration in colostrum should be > 50g/L to be classified as satisfactory (Gulliksen et al., 2008). Icelandic dairy cattle have been reported to have a lower colostral IgG concentration than the recommendation (Gulliksen et al., 2008; Kehoe et al., 2007; Pritchett et al., 1991; Quigley et al., 1994; Vagnsdóttir, 2018). There are several factors that influence the individual IgG concentration in colostrum. These factors include time passing from calving to milking of colostrum, and the parity of the cow (Gulliksen et al., 2008; Moore et al., 2005; Morin et al., 2010; Pritchett et al., 1991; Quigley et al., 1994). Although the quality of colostrum is important, a high IgG concentration in colostrum alone does not ensure high serum IgG levels in calves. The method of feeding, amount fed at first feeding, and age at first colostrum feeding are important colostrum management factors.

Feeding methods most frequently applied and described in studies, are feeding by oesophageal tube, by nipple bottle or by the calf suckling its own dam. Many studies have compared bottle feeding and suckling from the dam and have received different results (Franklin et al., 2003; Nocek et al., 1984; Quigley et al., 1995; Stott et al., 1979). However, in the studies reporting more positively of the suckling method, the nipple bottle fed calves only received between 0.5 and 2L of colostrum, which is known to be less than optimal (Besser et al., 1991). When comparing the use of oesophageal tube and nipple bottle, some studies found no significant difference between the efficacy of absorption when comparable amounts of IgG were fed through colostrum (Besser et al., 1991; Chigerwe et al., 2012). However, some studies have indicated that the use of an oesophageal feeder may be correlated with decreased efficiency of absorption of IgG (Hopkins & Quigley, 1997; McGuirk & Collins, 2004), but it allows for a more rapid feeding of a larger volume of colostrum, which has a positive impact on the immunization (Besser et al., 1991).

For volume of colostrum fed at first feeding, every additional gram of IgG per litre colostrum, lowers the risk of FPT (Lora, Barberio, et al., 2018). A minimum amount of total IgG to decrease the prevalence of FPT has been reported for Holstein calves, and to obtain this, most colostrum feedings need to consist of 3 to 4L (Besser et al., 1991; Chigerwe et al., 2012; Osaka et al., 2014). The timing of the first colostrum meal is also an important risk factor. Feeding colostrum within the first hour of life has been reported to show a significant difference compared to feeding 6 hours after birth (Fischer et al., 2018). Apparent absorption of IgG has been reported to fall from 65.8% at 6 hours to 11.5% at 24 hours (Matte et al., 1982), which is similar to what was reported by Rajala & Castrén (1995) that every 30 min delay decreases the amount of IgG absorbed. Lora, Barberio, *et al.* (2018) concluded that risk of FPT increases with every hour delay for the first feeding and in order to completely avoid

FPT, calves should be fed 2.5L of good quality colostrum within 1 hour of life. Reports have also shown that an additional colostrum meal is beneficial (Abuelo et al., 2021; Morin et al., 1997).

1.3 How to test for IgG

To investigate whether a calf has an adequate transfer of passive immunity, serum can be tested for IgG in several different ways. The most accurate way is using radial immunodiffusion (RID) (Akköse et al., 2022; Zakian et al., 2018). RID is however time-consuming, expensive, and unpractical for on-farm use (Elsohaby et al., 2015). For on-farm monitoring, other means like measuring serum total protein or the Brix%-value by digital refractometry are more practical. These methods are frequently used and have been thoroughly tested and validated as reliable in estimating serum IgG (Akköse et al., 2022; Hernandez et al., 2016; Zakian et al., 2018). The correlation coefficient (r) between serum Brix% and serum IgG measured by RID reportedly ranges from 0.74 to 0.93, indicating that using Brix% measurements instead of RID would generate comparable results (Akköse et al., 2022; Deelen et al., 2014; Elsohaby et al., 2015; Hernandez et al., 2016; McCracken et al., 2017; Morrill et al., 2013; Thornhill et al., 2015). Different cut-off values have been described for using Brix% to evaluate FPT, ranging from 7.8 to 8.4% (Deelen et al., 2014; Lombard et al., 2020; Morrill et al., 2013; Zakian et al., 2018).

1.4 When to test for IgG

It has been recommended to measure serum IgG in order to evaluate sick calves and perform risk assessments of individual calves, as well as to evaluate the management procedures on farms (Hancock, 1985). When testing the serum, calves must be within a certain age range to receive accurate results and studies have shown that the peak serum IgG concentration occurs > 24 hours after birth. Calves should therefore be > 24 hours old at sampling (Husband et al., 1972; Wilm et al., 2018). Endogenous production of IgG has been reported to start from around day 8 after birth (Husband et al., 1972), and because of the endogenous production, as well as catabolism of passive immunoglobulins, results can be obscured if calves > 7 days are sampled (Hancock, 1985). Others have reported a later beginning of immunoglobulin synthesis at around 4 weeks (Burton et al., 1989) and earlier endogenous production has been reported as a result of FPT or partial failure of passive transfer (Furman-Fratczak et al., 2011).

1.5 FPT status in Iceland

Despite improvements in this field, FPT is still a general problem in the modern dairy industry (Beam et al., 2009). Prevalence and risk factors of FPT have been abundantly researched in other countries, but due to sparse data available, knowledge about FPT in Icelandic cattle is still limited. A single study has been published by Oddsdóttir *et al.* (2022) on passive transfer of immunity in a limited cohort of calves in Iceland. The experiment included 11 Icelandic dairy calves from a single farm, and the mean serum IgG 24 hours after birth was 8.02g/L (± 5.0). Despite these low concentrations of serum IgG, calf mortality is relatively low compared to other northern European countries (Agerholm et al., 1993; Gulliksen, Lie, Løken, et al., 2009; Svensson et al., 2006). The average calf mortality (1-180 days) in Iceland is 2.9%, ranging from 2.6% to 3.1% from January to November 2022 (data currently available), and for each region of the country, calf mortality ranges from 1.9% to 5.7% in the same period (Ráðgjafamiðstöð landbúnaðarins, 2022).

1.6 Aims and objectives

Due to limited data available on the health status of Icelandic calves and a knowledge of lower composition of IgG in Icelandic dairy cow colostrum, different cut-off values for FPT than those used for other breeds of cattle could be necessary. Considering the lack of knowledge, this study aims to gather data on the health from neonatal Icelandic calves. The objectives were to determine how the IgG levels of these calves compare to recommended levels for other common dairy breeds and whether there are significant differences in estimated serum IgG levels amongst calves according to sex, age, method of first colostrum feeding, age at first colostrum feeding, and amount fed at first colostrum feeding. The necessary data was gathered through clinical assessments of calves, including weight, heart-girth measurement, serum Brix% measurement, and through interviews with farmers about colostrum management. The interviews were also used to test, whether the risk of FPT is contingent upon the on-farm colostrum management regarding method of first colostrum feeding as well as age at first feeding, and volume of first feeding.

2. Materials and methods

2.1 Literature search

The database used for the primary literature search was Ovid MEDLINE(R) ALL. The keywords used were variations of calf, cattle, bovine, cow, immunoglobulin G, transfer of passive immunity, and failure of passive transfer, and abbreviations were included. The keywords were combined using OR or AND. Language was limited to English and the Scandinavian languages and further screening for relevance was performed by reading the abstracts. The literature search was supplemented by the snowballing method, using Medline, web of science, and Scopus to recover potentially relevant articles. We eliminated most articles from before 1990 because of timely relevance, except those we considered of major importance.

2.2 Power analysis

To make an estimate of the required sample size to estimate a mean, a power analysis was performed. According to Houe *et al.* (2004) is it possible to calculate a sample size based on a mean of standard deviations found in literature when presuming the Brix%-value of calves follows a normal distribution. Equation used for this calculation is as follows: **n** is calculated sample size, $Z^2_{1-\alpha/2}$= 1.96, with the confidence interval is set at 95%, $\boldsymbol{\sigma}$ is the known standard deviation (SD), and **L** describes how much we can allow our results to deviate from our initial guess.

$$n = \frac{Z^2_{1-\alpha/2}\sigma^2}{L^2}$$

The Brix% levels described in various studies range from 8.2% to 9.1%, with SD ranging from 0.1 to 1.0 (Akköse et al., 2022; Elsohaby et al., 2019; Stojic et al., 2017; Sutter et al., 2020). The values used were SD (mean SD from literature) = 0.7, L = 0.2, α = 0.05 and according to the sample size calculation, the required sample size was 48 calves (Houe et al., 2004). However, as there is no available previous data on serum Brix% levels from Icelandic calves and only one similar study has been performed in Iceland with a very small sample size (Oddsdóttir et al., 2022) a larger sample size than calculated was considered appropriate. Hence, the application was for 75 calves, which was considered practical within the timeframe of the study.

2.3 Ethical approval

This study was approved by Matvælastofnun and carried out under license reference number 2208612, according to article 4 of law no. 55/2013 on animal welfare and regulation no. 460/2017 on the protection of animals used for scientific purposes.

2.4. Data

Convenience- and geographical sampling methods were used for this pilot study. This was primarily due to the distances that would be necessary to travel, to reach farms over the entire country, and due to the limited time available to conduct the sampling. Dairy farms in Iceland are mainly concentrated in a few areas, two of these areas being in the north/northwest and the south area of the country. By concentrating on these two areas, it was possible to include farms of different sizes and management styles in the study, while also not spending too much of the sampling period on transportation.

2.4.1 Farms and animals

A list of expected calvings was received from The Icelandic Agricultural Advisor Centre (RML), created from insemination- and pregnancy registrations made in the Icelandic Cattle Recording system, “Huppa”. The list included the farm name, registration number, and municipality. For the individual cows, the list included cow number, name, date of birth, parity number, and date of the next expected calving. All registered expected calvings from 01.09.2022 were on the list, but only expected calvings in the period from 26.09.2022 to 21.10.2022 were included in the study, as this was the chosen sampling period. Farms in excluded municipalities were removed from the list. Included municipalities were Skagafjörður, Húnaþing-vestra, Skagabyggð, Húnavatnshreppur, Akrahreppur, Dalvíkurbyggð, Eyjafjarðarsveit, Hörgársveit, Sveitarfélagið Árborg, Hrunamannahreppur, Ölfus, Grímsnes- og Grafningshreppur, Skeiða- og Gnúpverjahreppur, Bláskógabyggð and Flóahreppur. After implementing these exclusion criteria, the list consisted of 215 farms and 817 expected calvings.

By random selection, a total of 86 farms were contacted and thereof 37 were visited. Two of the farmers were not interested in taking part in the study, and the remaining 47 farms could not be visited due to lack of calves in the age group, geographical impracticalities, or because we had already reached the quota of 75 calves. Three municipalities were not represented in the final sample due to the same reasons; they were Akrahreppur, Dalvíkurbyggð, and Ölfus.

2.4.2 Data collection

At each farm, farmers or their substitutes were asked questions from a questionnaire. The questionnaire consisted of 15 close-ended questions with 2-5 response options for each answer. It was inspired by a survey from Norway conducted from 2004 to 2007 (Gulliksen, Lie, Løken, et al., 2009), which focused on calf- and colostrum management. Prior to the on-farm application the questionnaire was modified and sent to three Icelandic farmers to investigate the clarity and suitability of the questionnaire. One responded and gave the suggestion to add the question of whether calvings happen outside during the summer or not. This farmer was not included in the study. After data collection three questions from the questionnaire were in focus for further analysis. These were: (1) how are the calves fed after birth, (2) what is the maximum time for 90% of the calves from birth to first feeding, and (3) what amount of colostrum is offered at first feeding. Answer options were (1) suckling the dam, nipple bottle feeding, or bucket feeding, (2) < 2 hours, 2-4 hours, 4-6 hours, > 6 hours, and suckling the dam, (3) ≤ 1 L, 1.1-2 L, > 2 L, and suckling the dam.

As earlier accounted for, only calves from 1 to 7 days of age were included in the study. At the lower end of the time limit (i.e., first day of life), we asked the farmer at what time the calf was born to make sure it was at least 24 hours. At the higher end of the limit, we counted the days, and not hours, e.g., if a calf was born on October 1st, it was in the age category until and including October 8th. In order to investigate the calves' general health, the rectal temperature was measured, and the calves were scored for the following 12 clinical scorings: nasal discharge, ear- and head tilt, eye discharge, umbilical region, joints, weight-bearing, hair layer, diarrhoea, hair loss, body condition, soiling, and coughing. These scorings were performed using the same methods as in the project Robuste Kalve in Denmark (Pryds Klaustrup, 2021). Out of these clinical scorings, calves only scored in four categories. These four were nasal discharge (0 = no discharge, 1 = serous discharge, 2 = mucopurulent discharge), diarrhoea (0 = no diarrhoea, 1 = not watery but mixed with blood and/or mucous, 2 = watery and/or very mucoid and/or blood mixed), body condition (0 = normal, 1 = underweight, 2 = overweight) and soiling (0 = < 2 palms, 1 = > 2 palms, 2 = > 25% of the calf's surface area being soiled). Sex was noted for all calves, age for 72 calves, and 47 calves were weighed (kg) and had heart-girth circumference measured (cm). We chose to only measure the heart girth of calves that were also weighed. The calves were picked up and weighed on a bathroom scale and the one lifting the calf was weighed right before or after without moving the scale. Then the weight of the lifter was subtracted from the total weight. This was only done where the circumstances allowed for the scale to measure somewhat accurately, meaning that for example pens with deep bedding without access to a solid foundation, were excluded from the weighing.

2.4.3 Blood samples

Blood samples were collected from the calves using jugular venepuncture. The area was sanitised with alcohol prior to sampling. For the first 19 samples, 18G needles (BD Microlance ™ 3) and a 10- or 20-mL syringe (KRUUSE) were used and the blood was emptied into the serum tubes with gel (VACUETTE ® 8ml CAT Serum Sep Clot Activator) through the membrane immediately after sampling. After receiving vacutainers (VACUETTE ® QUICKSHIELD Complete, 21Gx1½) and serum tubes (VACUETTE ® 9ml CAT Serum Clot Activator) without gel, these were used for the remaining samples. In a few cases (2-3 times), if a sample was difficult to obtain using the vacutainer, a needle and syringe was used as in the beginning of the study. Following collection, all samples were placed upright in the car, where the temperature ranged from -2 to 10°C. The first 60 blood samples were centrifuged within 10 hours after collection. The centrifuge used for the first 23 samples was a Hettich zentrifugen EBA 12 1000 230V and for the rest a Heraeus Labofuge 300 Centrifuge was used.

The blood samples were kept upright at room temperature (approximately 20^{o}C) for 30 to 90 minutes before centrifuging at 4000-4500 rpm for 10 minutes (Cockcroft, 2015), with an additional 5 minutes if the sample was not quite separated or if the gel-clot was not in between the serum and plasma. Four samples had signs of haemolysis without deviating. Due to practical reasons, the remaining 15 samples were not centrifuged but left, upright at room temperature for 20-24 hours (Cuttance et al., 2017; Ramon Armengol, 2022) and by then the samples had a defined serum layer. The Brix%-value was measured with two digital Brix-refractometers (ATAGO PAL-1 3810 Digital Hand-held “Pocket" Refractometer, Serial no. ST010791/ST010761) with a range of 0-53%, resolution of 0.1% and an accuracy of +/-0.2%. Two identical refractometers were used to ensure any possible changes in either refractometer were detected and to confirm that they measured within the same range. Prior to the start of the study, a single 20mL blood sample was taken from one calf as a test sample. The sample was centrifuged according to the regimen above and 10 measurements were performed with each refractometer reset to zero in between each measurement. The measurements were no more than 0.2% apart, and mostly measured the same value or at 0.1% apart. When measuring the serum, approximately 0.3mL of serum (7-8 drops) were placed on the prism for each sample, using single-use pipettes. The serum was wiped off with a clean tissue, demineralized water dripped onto the prism, and wiped again. The refractometer was reset after every round of samples (at the end of every sample day).

2.5 Statistical analysis

Multiple farms were visited each sampling day. At the end of every sampling day, data from the questionnaires, clinical scorings, and calf measurements were written in Excel version 16.67 (Microsoft Corporation, 2018). Analysis of the data was performed in the statistical program R 4.2.2. (R Core Team, 2020) and RStudio (RStudio Team, 2020) as well as in Excel, with the significance level set at 0.05. Descriptive statistics were evaluated using the number of responses and percentages for categorical variables (questionnaire and clinical scorings). For continuous variables (weight, heart-girth measurement, rectal temperature, age, and Brix%) mean, SD, median and interquartile range (IQR) were used. The normality of continuous variables was assessed by plotting frequency histograms and using the Shapiro-wilk test. Variables that followed a normal distribution were heart-girth measurement and Brix%. Variables not normally distributed were weight, rectal temperature, and age. Pearson's correlation was used to evaluate the correlation of weight and heart-girth measurement. A parametric test was chosen despite not normal distribution of weight, where a best fitted line equation was asked as an outcome of this correlation testing. Homogeneity for Brix% and certain categorical variable groups (sex, age, feeding method, age at first feeding and amount at first feeding) were assessed by visualisation of box plots, where all variables showed difference in variability and/or median (heterogeneity).

2.5.1 Brix%

The level of agreement between Brix% measurements was determined using Bland-Altman plot and Pearson's correlation. To compare the Brix% with findings from other studies, the Brix% was categorised into four different levels. This was done based on a scale developed by Lombard et al. (2020), which uses the categories "poor", "fair", "good", and "excellent", for IgG levels of <8.1, 8.1-8.8, 8.9-9.3, and ≥9.4 g/L, respectively. Individuals in the "poor" category are classified as having FPT, while those included in the other variables are classified as having APT.

All models including Brix% and one of the categorical variables (sex, age, feeding method, age at first feeding and amount at first feeding) were non-parametric. Non-parametric tests were chosen, despite normal distribution of Brix%, instead of parametric where there was not an even distribution of calves among variable groups, and some groups had very few calves. This was also due to herd effect, which was not considered in the sample size calculation, and was caused by some farms only including one calf. Only the questionnaire was used to place calves in their respective management categories, hence information about managemental procedures for individual calves was not included. For one continuous outcome variable and one categorical explanatory variable a Mann-Whitney or Kruskal-Wallis model were used. Mann-Whitney when the explanatory variables were dichotomous (sex and feeding method) and Kruskal-Wallis model when the explanatory variables including more than two groups (age, age at first feeding and amount at first feeding). When testing if there was a significant difference in risk of FPT within different management groups (feeding method, age at first feeding and amount at first feeding) a Fisher Exact model was used to test univariable associations. Fisher Exact model was chosen where there was at least one variable that included less than five calves in all models.

3. Results

3.1 Farms

Of the 37 farms visited, all were free stall systems and 62% had a single cow calving pen. On 86% of the farms, milking 2 to 4 was used for new-born calves and on 89% of farms calves received colostrum from their own dam. Out of all farms, 3 farmers occasionally monitored the colostrum quality, one used a digital Brix refractometer, and two used colostrum hydrometers. Bucket feeding was never used to feed calves the first colostrum, but 92% of farms used nipple bottle feeding. A third of the farms feed 90% of their calves within 4 hours from birth and 8% leave the calf to suckle the dam. Almost two thirds (62%) of the farms provided between 1 and 2L of colostrum feed at first feeding. An overview is provided in Table 1 of the answers to the questionnaire considered the most pertinent factors for Brix% and FPT. Results from all the questions in the questionnaire can be seen in appendix 7.1.

Table 1: Results from farmers questionnaire

Question	Response options	Number	Percentage %
Stall type	Tie stall	0	0
	Free stall	37	100
Calving facilities	Tied	0	0
	Alley area	10	27

	Calving pen alone	23	62
	Calving pen more than one	4	11
Calving during summer	Always inside	18	49
	Sometimes outside	19	51
Separation from dam	Immediately (0-2 hours)	4	11
	During the first 24 hours	24	65
	After the first 24 hours	9	24
How are the calves fed the first colostrum after birth	Suckling the dam	3	8
	Nipple bottle feeding	34	92
	Bucket feeding	0	0
Tube feeding	Never	33	89
	Yes, always (almost always)	0	0
	Yes, when the calf does not suckle	4	11
Maximum time for 90% of calves from birth to first feeding	Less than 2 hours	8	22
	Between 2 and 4 hours	4	11
	Between 4 and 6 hours	15	40
	After 6 hours	7	19
	Suckles the dam	3	8
Amount of colostrum offered at first feeding	≥ 1 L	2	6
	1.1 – 2 L	23	62
	> 2 L	9	24
	Suckles the dam	3	8
Source of colostrum	Their mother	33	89
	Colostrum bank	1	3
	Suckles the dam	3	8
Is all colostrum used, independent on cow lactation number	Yes	36	97
	No	1	3
What happens to milking 2 to 4	To new-born calves	32	86
	To all calves receiving milk	5	14

	Other	0	0
Is colostrum quality checked	No	34	92
	Yes	1	3
	Sometimes	2	5

3.2 Animals

On average, there were 2.0 calves from each farm, ranging from 1 to 6 calves. One farm had 6 calves, while none had 5 calves. Included calves were between 1 and 7 days old with both days included, and the mean age was 4.2 (± 1.7) days. For rectal temperature, 50.7% of calves were below 39°C and no calf had rectal temperature over 40.5°C. The calves scored only on 4 of the 12 scores included in the study as already described in section 2.4.2. Results from clinical scoring and measurement of calves can be seen in Table 2, for the full list of clinical scorings, see appendix 7.2.

Table 2: Results from clinical scorings and measurements of calves.

Parameter	Score results	Proportion %	Mean (SD)	Median (IQR)	Min	Max
Nasal discharge	0: 73 1: 1 2: 1	0: 97.3 1: 1.3 2: 1.3				
Diarrhoea	0: 54 1: 19 2: 2	0: 72.0 1: 25.3 2: 2.7				
Body condition	0: 68 1: 7 2: 0	0: 90.7 1: 9.3 2: 0				
Soiling	0: 49 1: 19 2: 7	0: 65.3 1: 25.3 2: 9.3				
Weight (kg)			32.9 (4.67)	33.4 (5.3)	14.5	42
Heart-girth (cm)			79.7 (2.97)	80 (3.5)	72	86
Age (days)			4.2 (1.65)	4 (2)	1	7
Rectal temperature (°C)			39.0 (0.42)	39 (0.45)	37.8	40.3

Approximately 37% of calves had no clinical scoring and 27% of calves only scored on soiling, meaning 64% of calves had no sign of disease. Ten calves (13%) had more than one scoring and diarrhoea in combination with soiling was most the frequent and occurred a total of 6 times. A total of 36% of calves had signs of disease or were underweight, of which 78% had enteric signs. Only calves having score 2 in nasal discharge and diarrhoea are defined as being sick, in total there were 4% of calves scoring 2 in these two parameters. None of the calves included had received medical treatment. Mean heart-girth measurement was 79.7cm (ranging from 72 to 86cm) and mean weight was 32.9kg (ranging from 14.5 to 42kg). The smallest calf at 14.5kg weighed 10kg less than the second to smallest, the calf was dehydrated and very weak during the sampling and died later that day. Despite this calf's low weight it was included in all analysis. Heart-girth measurement was normally distributed ($p = 0.1$) (see Figure 1) while weight was not normally distributed ($p < 0.01$) (see Figure 2).

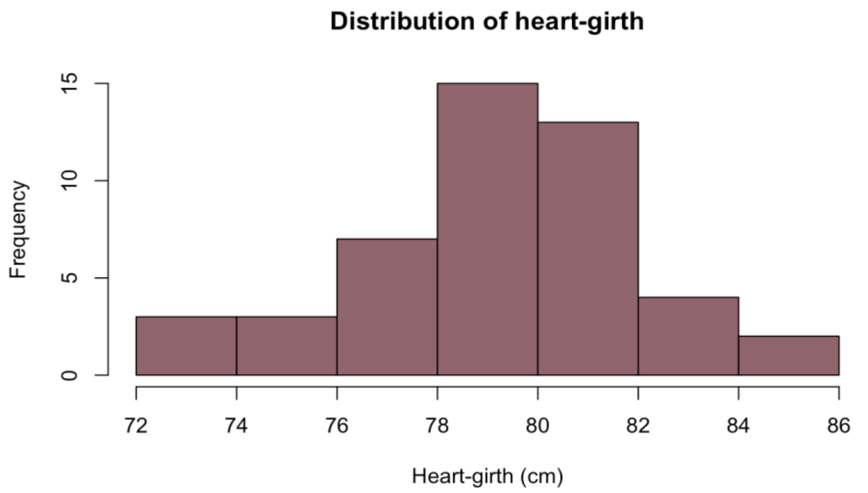

Figure 1: Histogram of heart-girth measurement (cm) showing a normal distribution ($p = 0.1$).

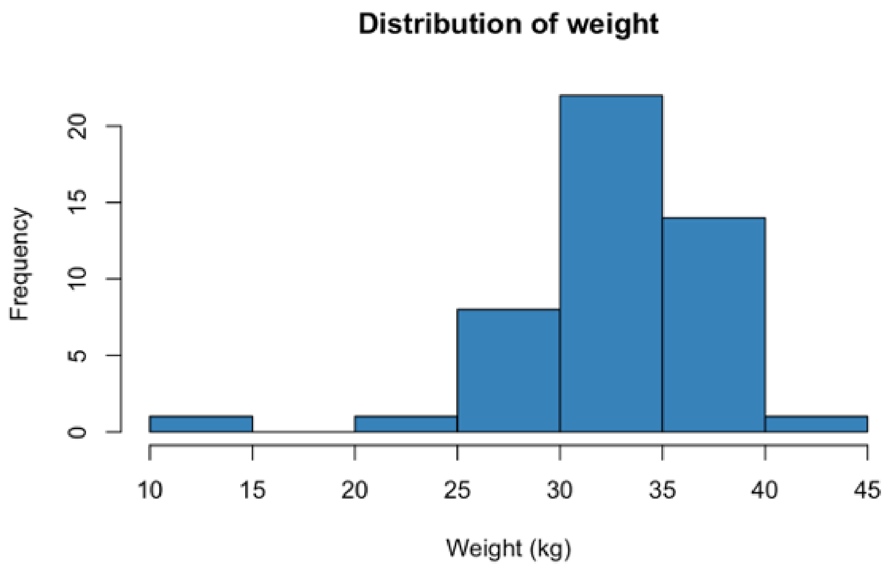

Figure 2: Histogram of weight (kg) showing a not normal distribution ($p < 0.01$).

These two measurements had significant linear correlation of R = 0.6 (p < 0.01) (see Figure 3), and equation for best fitted line is: $y(weight) = 0{,}9x\ (heart - girth) - 41.6$.

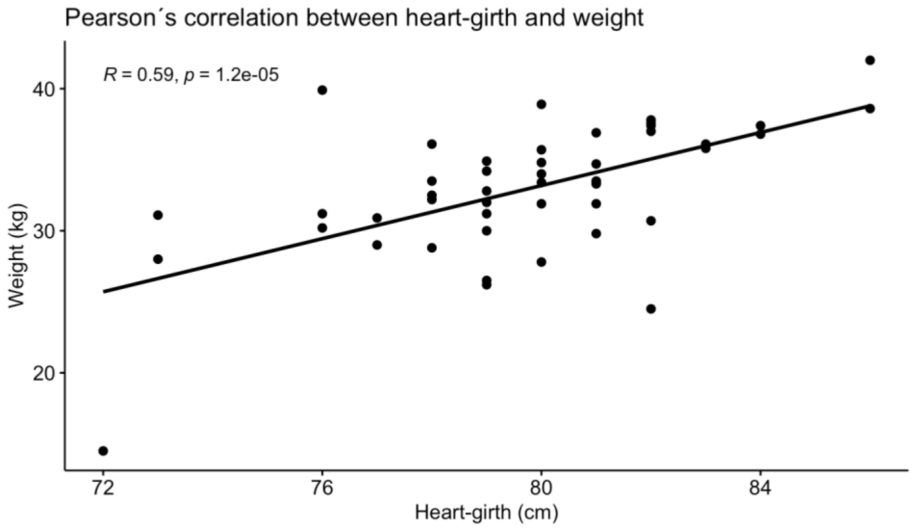

Figure 3: Pearson´s correlations graph for heart-girth (cm) and weight (kg). Significant correlation with R = 0.6 and p<0.01.

3.3 Brix%

The two Brix% measurements for each serum samples were highly correlated with R = 0.99 (p < 0.01), (see Figure 4), and an average difference for all samples was 0.016 with a 95% confidence interval (-0.13 to 0.16). For two calves the measurements were outside of the 95% confidence interval for difference. Figure 5 shows a Bland-Altman plot for the measurements' difference. Because of the high correlations of the Brix% measurements, it was assumed that the average of the two sets of measurements was representative of the sample population. The mean Brix% was made a new variable, named "Brix%", and will be used for further analysis from this point onward.

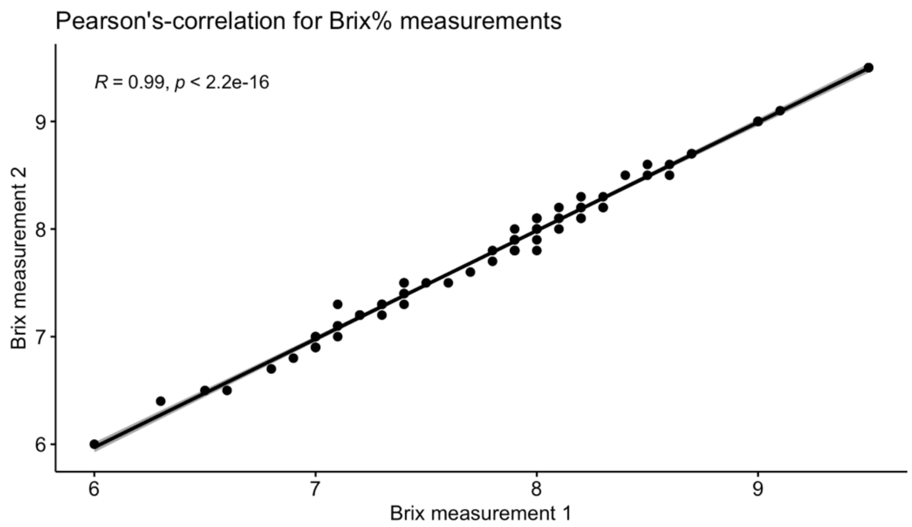

Figure 4: Pearson´s correlation between Brix% measurements 1 and 2. Significant correlation with R = 0.99 and p < 0.01.

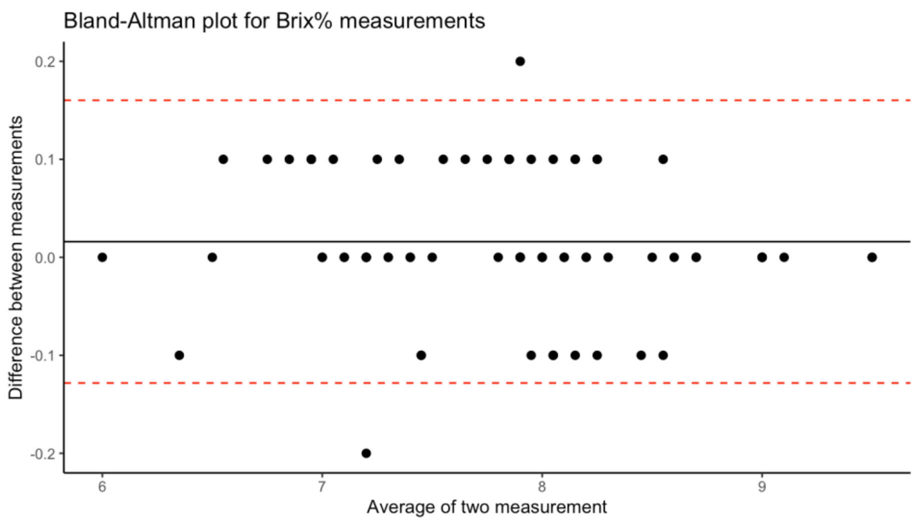

Figure 5: Bland-Altman plot for difference among Brix% measurements 1 and 2 where two samples show a difference out of the 95% confidence interval for the mean difference of measurements.

The Brix% ranged from 6 to 9.5%, and mean, SD, median and IQR for Brix% were 7.8, 0.7, 7.9 and 1%, respectively. The results were normally distributed with $p = 0.6$ (see Figure 6).

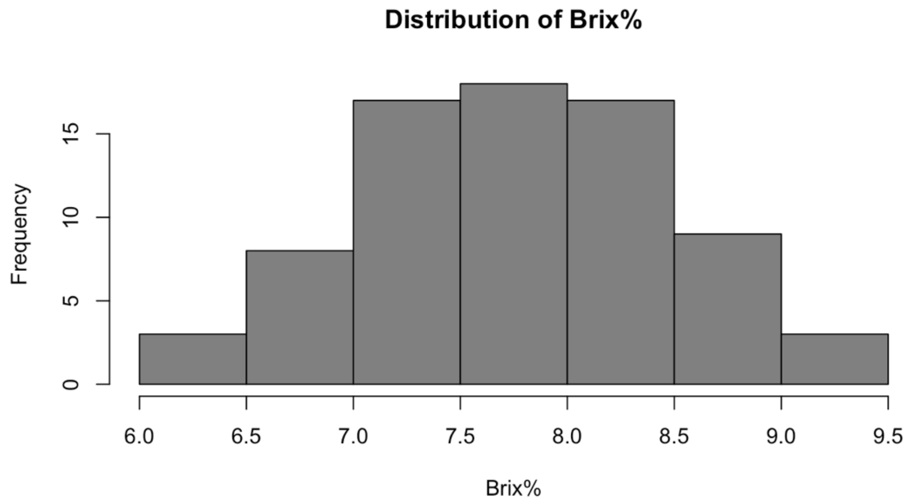

Figure 6: Histogram of Brix%, by using mean of both measurements for each calf. Brix% is normally distributed (p = 0.6).

For FPT two thirds of calves (66.7%) where in the category "poor", and one third (33.3%) of calves had APT. Percentage of calves in every category for Brix% are listed in Table 3.

Table 3: Overview for percentage of calves in each category for serum Brix% measurement, based on Lombart et al. (2020).

Category	**"Poor" (< 8.1%)**	**"Fair" (8.1-8.8%)**	**"Good" (8.9-9.3%)**	**"Excellent" (≥ 9.4%)**
Percent	66.7%	24.0%	6.7%	2.7%

3.3.1 Animal variables and Brix%

There was an uneven distribution of sex in the study population with 55% bulls and 45% heifers. Brix% results are listed in Table 4 and distribution can be seen in Figure 7. Mean Brix% is the same for both groups but their median is different. For bulls their median is higher than mean which could be explained by one outlier. There is not a significant difference (p = 0.8) within these groups in median Brix%.

Table 4: An overview of the Brix% results for calves in different sex groups.

Sex	**Number of calves (percent)**	**Mean Brix% (SD)**	**Median Brix% (IQR)**	**Min Brix%**	**Max Brix%**
Heifer	34 (45%)	7.8 (0.8)	7.6 (1.1)	6.4	9.5
Bull	41 (55%)	7.8 (0.6)	7.9 (0.7)	6	9

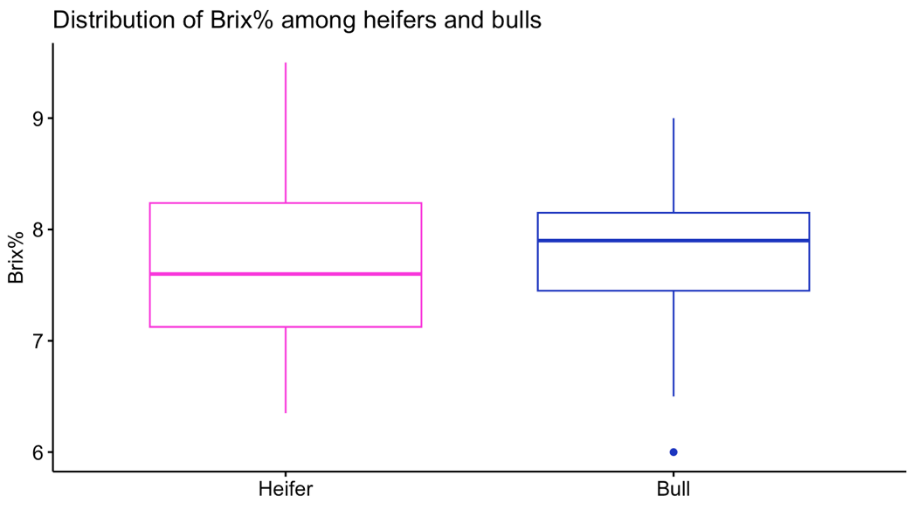

Figure 7: Distribution of Brix% grouped by sex. The box is drawn from first to third quartile and the vertical line goes through the box at the median, a point represents an outlier.

Most frequent ages among calves in the study population were 3 and 5 days, and the biggest range in Brix% was among calves at 4 and 5 days old. Five day old calves were also the ones with the highest mean and median. Brix% results for all age groups can be seen in Table 5. Distribution of Brix% can be seen in Figure 8. The age groups 5, 6 and 7 days old had outliers. Some variability is seen between the median Brix% in the age groups, but this is not statistically significant (p = 0.4).

Table 5: An overview of the Brix% results for calves at different age (days).

Age (days)	**Number of calves (percent)**	**Mean Brix% (SD)**	**Median Brix% (IQR)**	**Min Brix%**	**Max Brix%**
1	4 (5%)	7.5 (0.8)	7.3 (0.7)	6.9	8.7
2	7 (9%)	7.4 (0.7)	7.4 (0.9)	6.4	8.1
3	15 (20%)	7.7 (0.6)	7.5 (0.9)	7.0	9
4	14 (19%)	7.9 (0.8)	7.9 (1.0)	6.5	9.5
5	15 (20%)	8.0 (0.8)	8.2 (0.7)	6	9.5
6	10 (13%)	7.7 (0.6)	7.8 (0.7)	7	9
7	7 (9%)	7.8 (0.7)	7.9 (0.7)	6.6	8.6

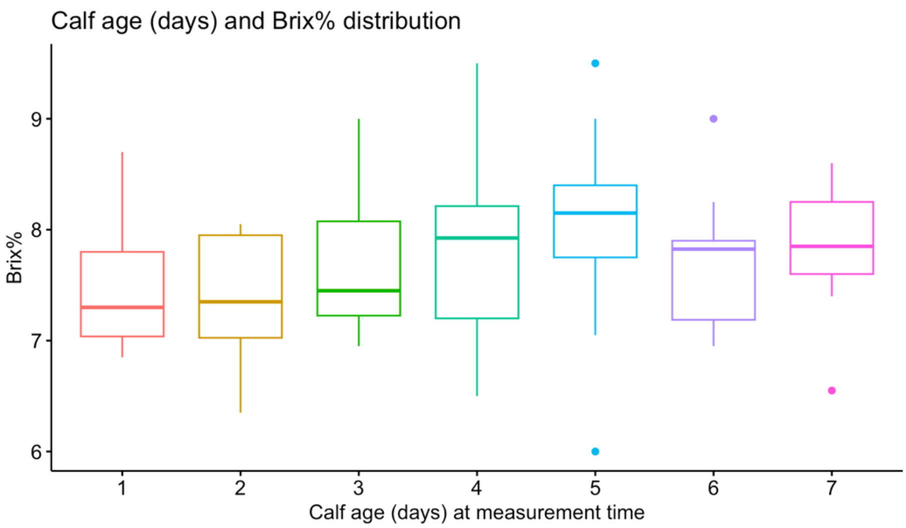

Figure 8: Distribution of Brix% grouped by age. The box is drawn from first to third quartile and the vertical line goes through the box at the median. A point represents an outlier.

3.3.2 Farm-management variables, Brix% and FPT

The feeding method of the first colostrum consisted of nipple bottle for 93% of the calves and the rest were left suckling the dam. These two groups had the same median for Brix% at 7.9, but the IQR was higher for the group suckling the dam (see Table 6). The distribution of Brix% in the two feeding method groups can be seen in Figure 9. There was not a significant difference (p = 0.6) in median Brix% between different feeding method groups.

Table 6: An overview of the Brix% and passive immunity results for calves that received first colostrum with different feeding method.

Feeding method	Number of calves (percent)	Mean Brix % (SD)	Median Brix % (IQR)	Min Brix %	Max Brix%	Number of calves with APT	Number of calves with FPT
Nipple bottle	70 (93%)	7.8 (0.7)	7.9 (0.9)	6.5	9.5	23	47
Suckling the dam	5 (7%)	7.4 (1.2)	7.9 (2.1)	6	8.5	2	3

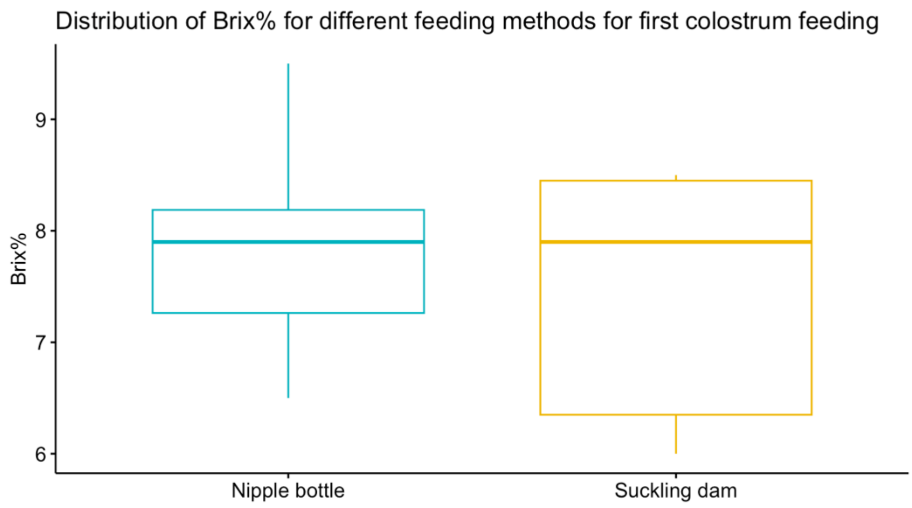

Figure 9: Distribution of Brix% grouped by different feeding method for first colostrum feeding. The box is drawn from first to third quartile and the vertical line goes through the box at the median.

There was not a significant difference ($p = 1$) in risk of FPT between the different feeding methods. The frequency of APT and FPT among different feeding groups can be seen in Figure 10.

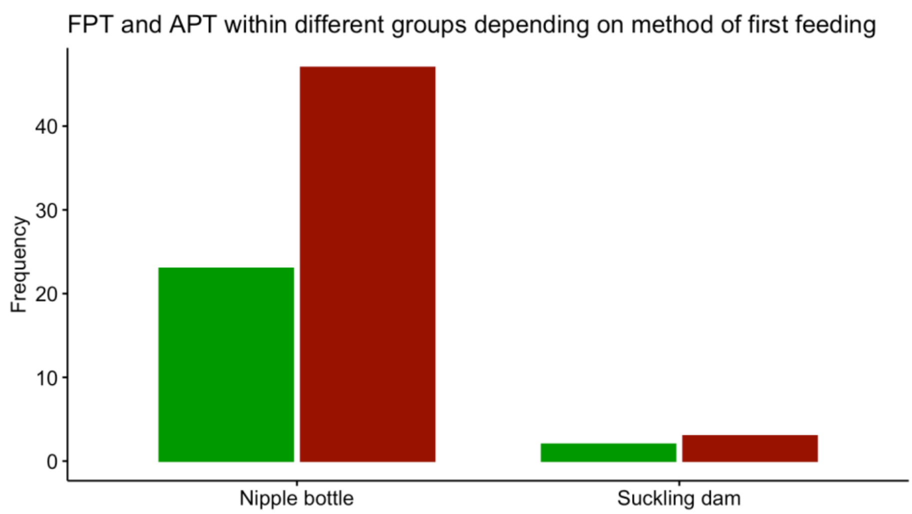

Figure 10: Frequency of FPT and APT grouped by different feeding method for first colostrum feeding. Green represents APT and red FPT.

Most frequent (45%) age at first feeding was 4 to 6 hours. Almost one third (30%) of calves had received colostrum within the first 4 hours after birth, 23% received colostrum within 2 hours and 7% were left to suckle the dam. Results of Brix% and passive transfer are listed in Table 7, Figure 11 presents the distribution of Brix%, and Figure 12 presents number of calves with APT and FPT among the groups of different age at first feeding. Calves fed within 2 hours from birth have the highest mean and median Brix%. Calves fed 4 to 6 hours after birth have the lowest median, but calves left suckling the dam have lowest mean. There was a significant difference ($p < 0.05$) in median Brix% between groups depending on age at first colostrum feeding. The significant difference was found between the groups of < 2 hours and 4 to 6 hours ($p < 0.01$). There was not a significant difference ($p = 0.2$) in risk of FPT between the different groups of age at first colostrum feeding, despite that, calves fed within 2 hours was the only group with higher a number of calves with APT than FPT.

Table 7: An overview of the Brix% and passive immunity results for calves that received first colostrum at different ages.

Age at first colostrum feeding	**Number of calves (percent)**	**Mean Brix% (SD)**	**Median Brix% (IQR)**	**Min Brix%**	**Max Brix%**	**Number of calves with APT**	**Number of calves with FPT**
< 2	17 (23%)	8.3 (0.7)	8.2 (0.8)	7	9.5	9	8
2 - 4	5 (7%)	8 (0.2)	7.9 (0.3)	7.8	8.3	2	3
4 - 6	34 (45%)	7.6 (0.6)	7.5 (0.9)	6.5	9	7	27
> 6	14 (19%)	7.8 (0.8)	7.6 (1.1)	6.9	9.5	5	9
Suckling the dam	5 (7%)	7.4 (1.2)	7.9 (2.1)	6	8.5	2	3

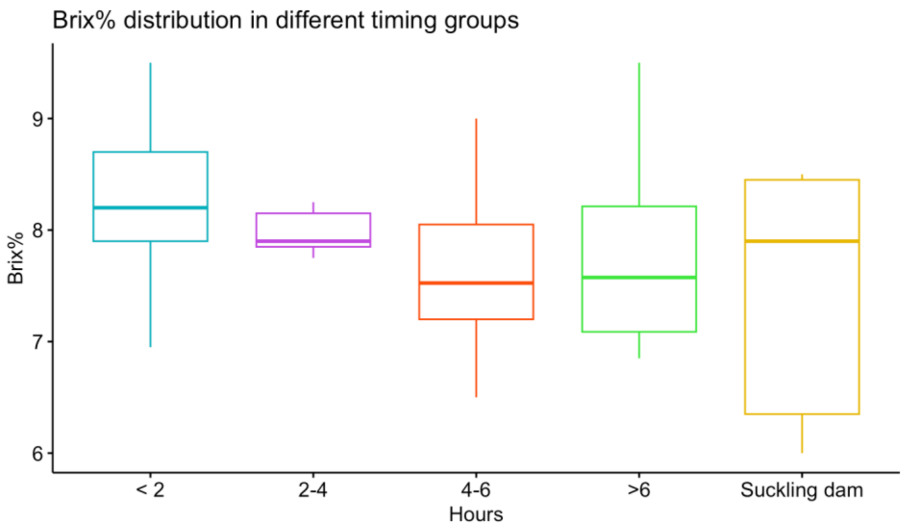

Figure 11 Distribution of Brix% grouped by different age at first colostrum feeding. The box is drawn from first to third quartile and the vertical line goes through the box at the median. A significant difference in median is between the groups < 2 and 4 to 6 hours with p < 0.01.

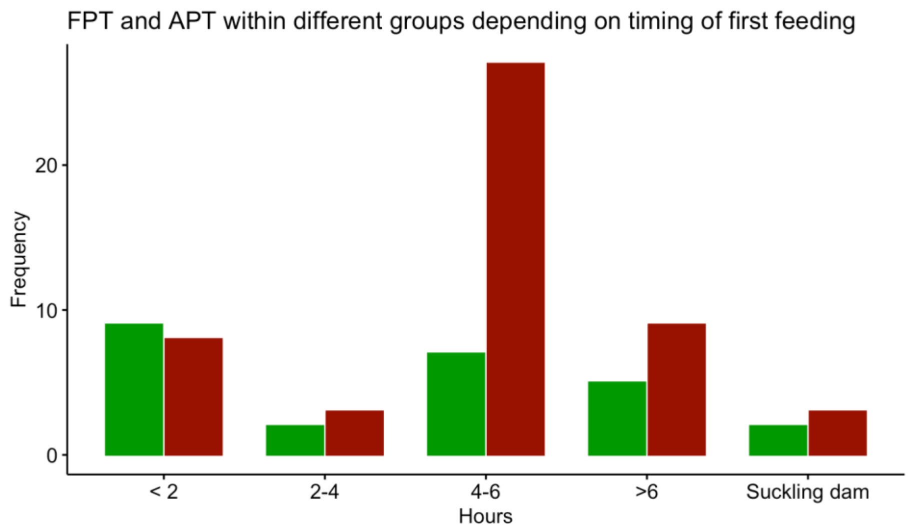

Figure 12 Frequency of FPT and APT grouped by different ages at first colostrum feeding. Green represents APT and red FPT.

The majority of calves (60%) received 1.1 to 2L of colostrum at first feeding. The calves receiving this amount of colostrum for the first feeding also had the highest mean and biggest range of Brix%. However, calves receiving ≤ 1L of colostrum had the highest median Brix% (8.1%). All results of Brix% and passive transfer are listed in Table 8. Figure 13 presents the distribution of Brix% and Figure 14 presents number of calves with APT and FPT among calves fed different amounts of colostrum at the first feeding. There was not a significant difference ($p = 0.3$) in median Brix% between different amounts of colostrum fed at first feeding. There is a proportionally higher frequency of FPT among calves receiving > 2L of colostrum than for the other groups. There was not a significant difference ($p = 0.7$) in risk of FPT between groups feed different amount of colostrum at first feeding.

Table 8: An overview of the Brix% and passive immunity results for calves that received different amount of colostrum in their first feeding.

Litres of colostrum in first feeding	Number of calves (percent)	Mean Brix% (SD)	Median Brix% (IQR)	Min Brix%	Max Brix%	Number of calves with APT	Number of calves with FPT
≤ 1	8 (11%)	7.8 (0.4)	8.1 (0.7)	7.2	8.2	2	6
1.1 - 2	45 (60%)	7.9 (0.7)	7.9 (0.9)	6.6	9.5	17	28
> 2	17 (23%)	7.6 (0.7)	7.5 (0.8)	6.5	9	4	13
Suckling the dam	5 (7%)	7.4 (1.2)	7.9 (2.1)	6	8.5	2	3

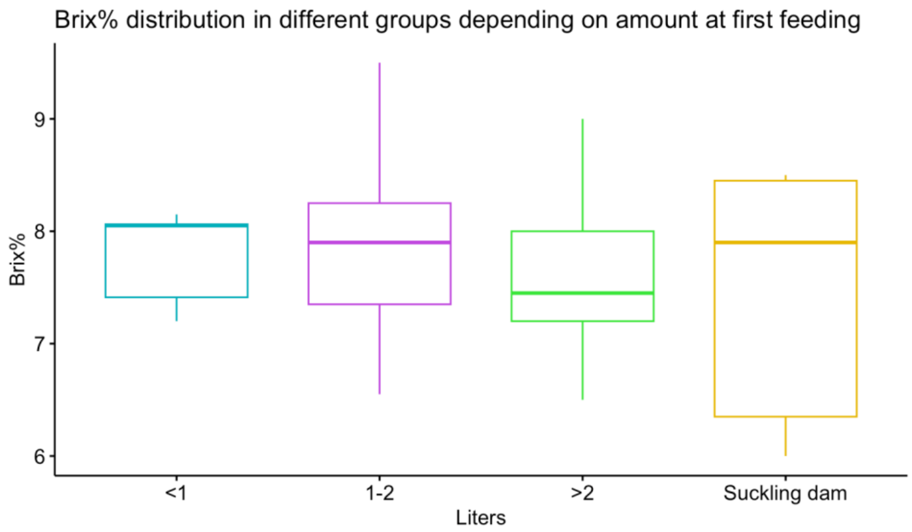

Figure 13: Distribution of Brix% grouped by different amount of colostrum fed during first feeding. The box is drawn from first to third quartile and the vertical line goes through the box at the median.

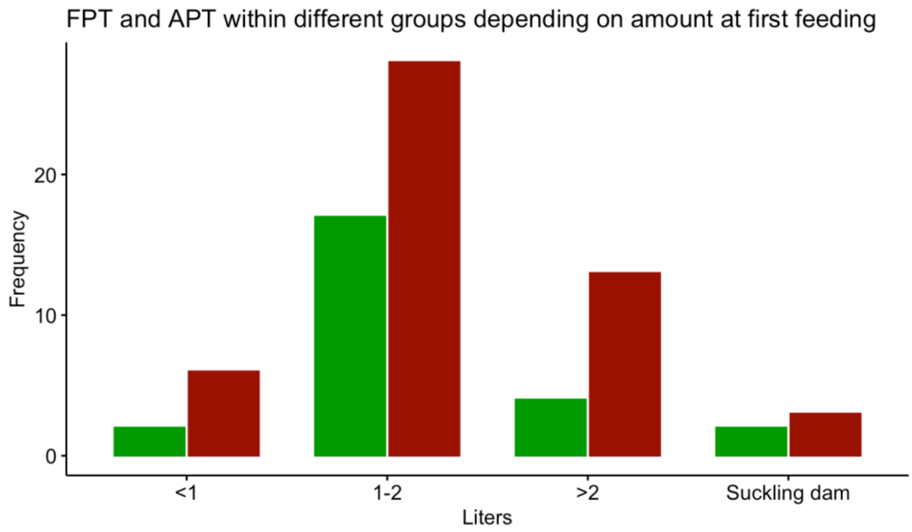

Figure 14: Frequency of FPT and APT for groups depending on amount of colostrum fed at first feeding. Green represents APT and red FPT.

In Figure 15 the variables "amount of colostrum" and "age at first feeding" have been plotted in a box plot against Brix%. The variable "suckling the dam" has been removed for both variables. Not all colostrum amounts are represented in all ages of first feeding and there is not an equal distribution in these groups. The box plot indicates that there could be a difference in Brix% depending on the combination amount and time of first feeding of colostrum. It further suggests that giving > 1L of colostrum within 2 hours after birth provides best results for Brix%. The statistical significance has not been calculated because of the risk that the earlier mentioned herd effect would absorb all the variation, because of the low number of animals included from each farm.

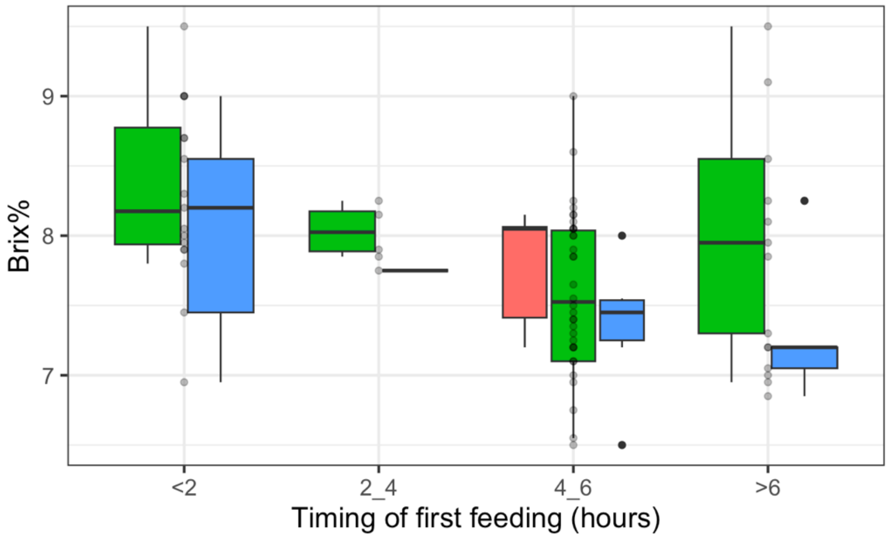

Figure 15: The plot shows the combination of amount of colostrum and age at first feeding against Brix%. The colours represent different amount fed at first feeding, pink represents <1L, green 1-2L and blue over 2L.

4. Discussion

With the aim to gain knowledge on the health status among neonatal Icelandic dairy calves, clinical examinations as well as interviews with farmers were conducted. Results of this research indicate that the calves are relatively healthy, with 4% presenting with signs of disease, 1.3% with respiratory- and 2.7% with enteric signs. However, the mean Brix% was 7.8% and merely 33.3% of calves had APT. An equation was presented in the study for linear correlation between weight and heart-girth measurement, which was of significance. There was significant difference in Brix% and age at first colostrum feeding between the groups < 2 hours and 4 to 6 hours ($p < 0.01$).

4.1 Farms

The farmer's management of colostrum and neonatal calves is essential for the calves' ability to absorb IgG (Weaver et al., 2000). The results from the questionnaire indicate that on 86% of farms, milking 2 to 4 is used for feeding neonatal calves. Feeding transition milk (milking 2 to 4) for 3 days after birth has been reported to increase the growth rate of calves through the preweaning period (van Soest et al., 2020). In order to make this more manageable, SEGES (a Danish agricultural advisor) recommends that excess colostrum and transition milk is stored in a milk bank. Thereafter 500-1000mL can be supplemented into a calf's usual milk feed, until the calf reaches 14 days of age (Martin, 2022). A limitation of this study is that the questionnaire did not include a question on whether farmers feed colostrum to calves more than once. This information might have been beneficial as numerous studies report that providing additional colostrum is preferable to one colostrum feeding. According to Abuelo *et al.* (2021) calves receiving two good-quality colostrum meals, the first within 2 hours of life and the second 5 to 6 hours later, have four times lower risk of FPT. Furthermore, these calves have lower pre-weaning morbidity, greater average daily gain pre-weaning, and a greater first lactation milk yield. Morin *et al.* (1997) report that a large volume (4L) of good quality colostrum should be fed within 3 hours of birth. However, if the quality is poor, it is advisable to administer two feedings of 2L within 6 hours of birth. Conversely, a study by Hopkins & Quigley (1997) showed no significant difference in whether calves received the total amount of colostrum over one or two meals. Data suggest that the efficiency of absorption in the second meal at 12 hours of age was similar to the first meal given shortly after birth, even though the efficiency of absorption declined over the first 24 hours of life. However, it is possible that the efficiency of absorption of the first feed was compromised, possibly by saturation of absorptive sites in the intestines. This might have caused a lower IgG absorption, than what could be possible from the IgG concentration in the colostrum (Besser et al., 1991; Hopkins & Quigley, 1997).

In the present study, 89% of farmers gave colostrum directly from dam to calf. This is comparable to a study by Lora, Gottardo, *et. al.* (2018), where calves were fed colostrum from their own dam on 86% of farms. This is likely beneficial, as higher serum IgG concentrations have been reported in calves receiving colostrum from a single dam, their own or otherwise, compared to calves receiving pooled colostrum (Barry et al., 2022). In the present study, only one farm (3%) used pooled colostrum for the newborn calves, indicating that this might be an uncommon managemental procedure in Iceland. Some results from the present study's farm-management questionnaire were compared to results from a Norwegian study by Gulliksen, Lie, & Løken (2009), which is the same study that was used as inspiration when designing the questionnaire. Calves suckling directly from the dam was reported on 8% of farms in this study, and similarly, the Norwegian study reported that 10.4% of farms used this feeding method. Likewise, the percentage of farms providing 1.1 to 2L of colostrum in the first meal correlated well in the present and the Norwegian study, at 62% and 64%, respectively (Gulliksen, Lie, Løken, et al., 2009). However, in this study, solely 22% of farms fed the first colostrum within 2 hours from birth, while 68.6% were reported by Gulliksen, Lie, & Løken (2009). Similarly, 33% of farms in the present study fed colostrum within 4 hours, compared to 78.4% in the Norwegian study (Gulliksen, Lie, Løken, et al., 2009). When comparing the results of the present study with both Gulliksen, Lie & Løken (2009) and Lora, Gottardo, *et. al.* (2018), it is apparent that there is a great likeness in the colostrum management regarding feeding method and amount of colostrum fed in the first feeding. Nevertheless, there seems to be room for improvement amongst the Icelandic dairy farmers concerning the age at the first colostrum feeding. The awareness of the importance of colostrum management amongst Icelandic farmers is varied, but most feed colostrum to calves from their own dam and use the transition milk for neonatal calves. However, the importance of feeding the first colostrum meal early, with colostrum of good quality and a high enough volume, seems unrecognised in many farmers. Some general guidelines might be of help in order to improve the colostrum management on some farms, as well as education of the farmers, so they can get a better knowledge on the profits of having calves with high levels of IgG.

4.2 Animals

In this study, 36% of the calves had clinical symptoms, in which 4% exclusively scored on body condition. As the cut off for clinical disease was limited to scores of 2, 4 % of calves were classified with disease. A study by Gulliksen, Lie, & Løken (2009) reports morbidity of 8.7% in calves up to 180 days, and Urie *et al*. (2018a) report morbidity of 33.8% in calves from birth to weaning.

Out of the 36% of calves with clinical symptoms in this study, 78% had enteric signs, while 8.3% has respiratory signs. Similarly, out of all affected calves, Gulliksen, Lie, & Løken (2009) report enteric- and respiratory signs at 43.7% and 33.3%, respectively, and Urie *et al.* (2018a) report that 56% of morbidity cases had enteric signs. Icelandic calves have low morbidity in comparison to those in the two studies referred to above. However, the age-gap is quite different, and therefore it might not be the most accurate comparison. In case of calf mortality, data is more parallel. Calf mortality in Icelandic calves from 1 to 180 days is 2.9%, compared to 3.3% in Norway for calves from 0 to 180 days (not including stillbirths), and 3.1% in Sweden for calves from 1 to 91 days (Gulliksen, Lie, Løken, et al., 2009; Ráðgjafamiðstöð landbúnaðarins, 2022; Svensson et al., 2006). As mortality rate and FPT have been reported to be positively correlated (Lora, Gottardo, et al., 2018), it might be assumed that Icelandic calves with this relatively low mortality rate, have high levels of Brix%. Yet, this is not the case in the current study, with a mean Brix% at 7.8% and 0.3% below the cut-off value for FPT. In this case, it should be considered that a definition of FPT should always be defined by health outcomes, as stated by Johnsen *et al.* (2019).

For this study, respiratory signs (nasal discharge) were only seen in two of the calves, and the discharge was only classified as mucopurulent (score 2) in one (1.3%) calf. Gulliksen, Lie, & Østerås (2009) reported the median age of respiratory disease to be 37 days. Similar results are reported in another study from Norway, as well as from Sweden and Denmark, where pneumonia is the most common cause of death in dairy calves older than 30 days (Agerholm et al., 1993; Gulliksen, Lie, & Østerås, 2009; Svensson et al., 2006). This, combined with the statement of Harðarson (2021) that respiratory disease is uncommon in Icelandic calves, suggests that calves in the 1 to 7 day age group are too young to be showing many signs of disease, especially the youngest calves in the study. This is due to the incubation time of the pathogens (Smith et al., 2020).

Studies from Norway, Sweden and Denmark have reported that for neonatal calves (< 30 days old) enteritis/diarrhoea is the predominant cause of death (Agerholm et al., 1993; Gulliksen, Lie, & Østerås, 2009; Svensson et al., 2006). In the present study two calves (2.7%) had diarrhoea, and in comparison, Hang *et al.* (2017) report a 17.5% occurrence of diarrhoea among calves up to two weeks of age. Fewer pathogens are known to cause enteric disease among Icelandic calves (Harðarson, 2021), and for example both rota- and coronavirus, which are recognized as two of the major pathogens associated with diarrhoea in calves (Smith et al., 2020) do not usually occur in Iceland (Harðarson, 2021). *E. coli* is the most common cause of diarrhoea amongst young Icelandic calves and can infect calves from birth (Harðarson, 2021) through faecal-oral transmission (Smith et al., 2020). In this study, the most frequent combination of scorings was between diarrhoea and soiling, but for the 2 calves having diarrhoea, none of these scored in soiling. This implies, that soiling is not a risk factor for diarrhoea. According to Harðarson (2021), the course of illness caused by *E. coli* can cause rectal temperature below the normal range. In the present study, 50.7% of calves had a rectal temperature below 39°C, which is the lower limit in the normal range according to Smith *et al.* (2020). Some of the calves were housed under suboptimal conditions, e.g., alone or in pairs in large pens, with wet or minimal bedding, and in some cases in draught. This might also contribute to lower rectal temperatures, as neonatal calves can have issues with thermoregulation (Nagy, 2009). Yet again it must be considered that the normal range in temperature is not specified for Icelandic calves and could differ from the calves used to set the range, even though this is considered unlikely. To gain additional knowledge on the health status of Icelandic calves, a more extensive study on similar clinical registrations from calves would be appropriate.

The mean weight of the calves in this study was 32.9kg, which is approximately 6kg to 10kg lower than reported in Holstein calves (Chigerwe et al., 2009; Osaka et al., 2014). This is probably due to the breed difference as the Icelandic dairy cattle is a breed highly genetically distinct from Holstein, as has already been established (Gautason et al., 2020). In 1992 an equation for converting heart-girth measurement to weight in Holstein calves was presented (Heinrichs et al., 1992). The calculated correlation between heart-girth and weight based on the gathered data in the current study was around 0.3 lower than presented by Heinrich *et al.* (1992), where the correlation was $R^2 > 0.9$. The equation was presented in the results section. The equation could become a useful tool for farmers to get an indication of weight based on heart-girth measurements. However, a larger sample size and/or a narrower age-gap is considered necessary to increase the accuracy of the equation.

4.3 Brix%

In the present study, 66.7% of calves had FPT when using the 8.1% Brix% cut-off. When using 10g/L as the cut-off value, a study from Iceland including 11 calves had 82% calves with FPT, leaving only 18% of calves with APT (Oddsdóttir et al., 2022). When comparing these two studies, it should be considered that in Oddsdóttir *et al.* (2022), IgG was measured by ELISA, while this study used Brix%, which is a measurement of total solids, not a direct measurement of IgG. There is no available data on correlation between serum Brix% and IgG in Icelandic calves, hence this would be ideal to investigate in a future study.

The prevalence of FPT ranges from 12% to 40.5% in various studies measuring Brix% or IgG, with cut-offs at 8.1% or 10mg/mL, respectively, and in some studies, both methods were used (Aleri et al., 2021; Beam et al., 2009; Bragg et al., 2020; Denholm et al., 2021, 2022; Elsohaby et al., 2015; Shivley et al., 2018; Urie et al., 2018b). A prevalence of FPT at 14.2% was obtained with RID by Denholm *et al.* (2021), but a vastly higher prevalence at 40.5% was obtained when using Brix%. Aleri *et al.* (2021) had a more modest difference, with an FPT prevalence at 8.7% and 13.1% using RID and Brix%, respectively. These results indicate that by using Brix% as a measurement for FPT, the percent of FPT might be overestimated, as has been reported by Denholm *et al.* (2021). Another possible overestimation of Brix% can be caused by dehydration (Cockcroft, 2015). As 28% of the calves included in this study had loose stools or diarrhoea, it is likely that several were dehydrated to a certain degree. Evaluation of hydration status was, however, not included in the current study and it is therefore not possible to conclude, whether this factor influenced the results. Furthermore, the study by Oddsdóttir *et al.* (2022) was performed during the winter (January to March), while the current study was performed during autumn (September to October). A seasonal variable in colostrum quality has been reported in Norway and Sweden, where farmers are obligated to keep the cows outside for at least 8 weeks from May to September (Gulliksen et al., 2008; Johnsen et al., 2019), which is also the case in Iceland (*Reglugerð Um Velferð Nautgripa*, 2014). A study by Gulliksen *et al.* (2008) has reported a seasonal effect, causing lower IgG levels in colostrum during the winter months (December to February), and this same effect might also be present under Icelandic conditions.

The mean Brix% for this study at 7.8% was about 1.4% lower and with a narrower range than reported by Deelen *et al.* (2014) at 7.3% to 12.4% (mean 9.2%) and a study from Vietnam found mean Brix% to be 9.3% within 80 calves (Hang et al., 2017). There have been reports of correlations between the IgG concentration in colostrum and the occurrence of FPT in neonatal calves (Johnsen et al., 2019). The low Brix% mean for calf serum correlates well with earlier findings in Iceland related to IgG in serum and colostrum, where Vagnsdottir (2018) reported a mean colostrum IgG of 26,83 g/L (± 23,83 g/L) from 114 cows, and Oddsdóttir *et al.* (2022) reported a mean of 11.54g/L from 11 cows. There are several factors that influence the individual IgG concentration in colostrum, including time after calving and parity. Morin *et al.* (2010) showed that the concentration of IgG decreased by 3.7% every additional hour after calving and for every additional litre of mammary gland secretion. The same study also found no linear relationship between the total mass of IgG in colostrum and time after calving, indicating that the IgG is diluted because of the increasing volume and not because of a decrease in the mass of IgG (Morin et al., 2010). Moore *et al.* (2005) got the same results with a reduction of IgG concentration during the first hours after calving, with a significant difference between hours 2 and 6. Hence, one reason for a relatively low IgG-concentration in colostrum of Icelandic cows could be delayed milking of colostrum, although this was not examined in the current study. Furthermore, the lacking information on the specific composition of components in colostrum of Icelandic dairy cows means, that it cannot be denied, that they might naturally have a lower level of IgG than other common dairy breeds. It should be kept in mind, that this discussion on colostrum from Icelandic cows is based on only two studies with 125 individuals in total. Therefore, further investigations should be made in order to make serious conclusions on the subject.

In the current study, two farms occasionally monitored colostrum using colostrum hydrometers, which have been reported to have low sensitivity, especially in samples with an IgG concentration below the cut-off at 50g/L, meaning that calves might be fed poor-quality colostrum even after monitoring (Pritchett et al., 1994). Since the Icelandic study reported a mean concentration below 50g/L, receiving falsely elevated colostrum immunoglobulin measurements is a potential risk faced by Icelandic farmers. A significant effect of the temperature of colostrum has been reported (Mechor et al., 1991), which would necessitate measuring the temperature of the colostrum before using the hydrometer, giving the farmer an extra step in the monitoring process, which could make it less likely that the farmer makes this a priority in the management protocol. A single farmer in this study used Brix refractometry on colostrum.

Comparing colostrum and serum Brix% for individual cow and calf couples might be interesting to study in Icelandic cattle, as the isolation of the breed might have caused a decreased pathogen load, naturally giving Icelandic cattle lower levels of IgG in serum and colostrum.

4.3.1 Animal variables and Brix%

In the present study there was no significant difference in median Brix% in the calf-level variables sex and age. Likewise, Burton *et al.* (1989) found no significant difference in serum IgG between heifer- and bull calves (p = 0.9) when serum IgG was measured 24-36 hours after birth and Aleri *et al.* (2021) found no associations between age or sex and risk of FPT when IgG was measured in 2 to 7 days old calves. Similarly, both Beam *et al.* (2009) and Denholm *et al.* (2022) included calves between 1 and 7 days old and did not find a significant difference in risk of FPT or Brix% in the age group. These findings seem to support the range in age chosen for this study. Shively *et al.* (2018) stated that serum IgG decreased by 0.7g/L for each day from birth for calves at 24 hours to 7 days old, and Barry *et al.* (2019) got similar results with a 1.28g/L (20%) reduction in serum IgG every day in the first 1 to 6 days from birth. In the present study, the highest mean Brix% was found in the 5 day old calves, meaning the daily decrease in Brix% was not seen in this case. However, it is important to note that this study had high farm-level effects, which were not accounted for during sampling and several farms only had a single calf in the right age. For a better evaluation of the effect of age on Brix%, a repeated serum sample from the same calf, or samples from younger or older calves on the same farm, should be performed.

4.3.2 Farm-management variables, Brix% and FPT

Regarding farm-management factors and their relation to Brix% and FPT, this study found significant differences among the age at first feeding and Brix%, but not for feeding method or amount fed at first meal. However, having a low percentage of calves suckling the dam gives the study a limitation in the ability to evaluate the effectiveness of this method. Although, looking at other studies, suckling and feeding by nipple bottle have been compared. Calves fed by nipple bottle have been reported to have lower serum levels of IgG (Quigley et al., 1995; Stott et al., 1979) and get more aggressive cases of diarrhoea (Bilik et al., 2013) than suckling calves, however, others contradict these statements reporting a higher risk of FPT in suckling calves (Beam et al., 2009; Besser et al., 1991; Lora et al., 2019). Franklin *et al.* (2003) and Nocek *et al.* (1984) compared these two feeding methods and found that calves fed by nipple bottle had better transfer of passive immunity and higher serum IgG, when measured 24 hours after birth. Leaving a calf to suckle the dam can, as well as timing and volume, affect the absorption of IgG. Suckling therefore leaves the calf at risk of FPT (Besser et al., 1991) and calves should always be assisted to suckle the dam as soon as possible, if this methos is to be used (Rajala & Castrén, 1995).

In the present study, there was a significant difference in Brix% among calves fed within 2 hours after birth and calves fed 4 to 6 hours after birth. These results are similar to those reported by Fischer *et al.* (2018), who reported a significant difference in serum IgG among calves fed 45 minutes after birth or 6 hours after birth. There was also a significant difference between feeding 45 minutes or 12 hours after birth, but not between 6 hours and 12 hours after birth. Rajala & Castrén (1995) reported that with every 30 min delay in colostrum feeding, the amount of IgG absorbed decreased by about 2mg/mL. Shivley *et al.* (2018) reported that for each hour delay following birth to colostrum feeding, serum IgG decreased by 0.32g/L. A limitation to this study is, that the number of calves in each of the categories in age at first feeding is not equal, i.e. most farmers fed the calves in the same time interval. Few calves were fed later than 6 hours after birth making a direct comparison difficult. This may have influenced the fact that there was no significant difference in Brix% for calves fed before 2 hours and after 6 hours. Both Beam *et al.* (2009) and Chigerwe *et al.* (2009) report that feeding colostrum later than 4 hours after birth is related to FPT. Lora, Barberio *et al.*(2018) report that risk of FPT increases by 13% with every hour of delay until the first colostrum feeding. So, despite not finding a significant difference in risk of FPT between different ages at first feeding in this study, having higher percentages of APT than FPT among calves fed within 2 hours indicates a lower risk of FPT in this category.

There was no significant difference in median Brix% among different groups for amount at first feeding. This contrasts with findings in other studies which have reported that feeding an additional litre of colostrum at the first feeding, can decrease the risk of FPT with 59% (Lora, Barberio, et al., 2018) and increase serum IgG with 0.57g/L (Shivley et al., 2018). The present study notes a higher percentage of calves with APT among calves fed 1.1 to 2L of colostrum, than calves fed > 2L of colostrum. Despite this there is not a significant difference in risk of FPT among the two groups. However, the amount at first feeding and its effect on Brix% is presumably influenced by other factors like the quality of colostrum fed and the calf’s weight (Beam et al.,2009). The colostrum quality is an unknown factor in this study and cannot be concluded upon. The multivariable analysis visualised in the boxplot in section 3.3.2 includes Brix%, amount at first feeding and age at first feeding. It shows that the only group with a median over the cut-off value of 8.1%, is the group of calves fed within 2 hours after birth. However, the range of Brix% for calves fed 2L within 2 hours is lower than for calves fed 1 to 2L within 2 hours. It would be expected that the calves fed the higher volume within the same timeframe would have the higher Brix% value, therefore this is an interesting find. Calculation for this multivariable analysis was not performed because the herd effect is too large and was not considered when calculating the study population. For further investigation, a study with more calves from each farm, hence considering the herd effect, is required.

5. Conclusion

The health of 75 Icelandic dairy calves from 1 to 7 days of age was assessed through clinical examinations, serum Brix% measurements, and interviews with farmers on 37 different farms. The calves are apparently in good general health, as there was a low occurrence of disease at 4%. Diarrhoea had the highest prevalence among calves with disease, while respiratory signs were more infrequent. The mean Brix% was 7.8%, which is low compared to reports from other countries and is below the cut-off for FPT at 8.1%. The prevalence of FPT was 66.7%, which is lower than previously reported for Icelandic dairy calves but is high in comparison to numerous reports from other countries. There was no significant difference between calf-level variables and Brix%. Age at first feeding in the groups < 2 hours and 4 to 6 hours was the only farm-level variable with significant difference ($p < 0.01$) to Brix%. There were no significant differences in risk of FPT and any of the tested risk factors, however the age at first feeding might have affect the risk of FPT and only calves fed colostrum within 2 hours of life had higher frequency of APT than of FPT.

Icelandic calves weigh less than some other common dairy cow breeds. The correlation between weight and heart-girth measurement was significant ($p < 0.01$), although lower than for the corresponding equation for Holstein calves. The equation seems to be a promising method for farmers of estimating the weight of Icelandic calves. The awareness of the importance of colostrum management amongst Icelandic farmers is varied, and compared to other countries, Icelandic dairy calves receive colostrum too late and in a too small amount. Steps should be taken towards improving managemental procedures on the farms as well as the farmers' understanding of the subject on colostrum.

Generally, further testing and larger sample sizes are required to make conclusions on the health and immune status of Icelandic dairy calves. This should include testing of the correlation between Brix% and direct measuring methods of IgG on serum.

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7. Appendix

7.1 Farmers questionnaire

Question	Response options	Number	Percentage %
Stall type	Tie stall	0	0
	Free stall	37	100
Calving facilities	Tied	0	0
	Alley area	10	27
	Calving pen alone	23	62
	Calving pen more than one	4	11
Calving during summer	Always inside	18	49
	Sometimes outside	19	51
Separation from dam	Immediately (0-2 hours)	4	11
	During the first 24 hours	24	65
	After the first 24 hours	9	24
How are calves fed the first colostrum after birth	Suckling the dam	3	8
	Nipple bottle feeding	34	92
	Bucket feeding	0	0
Tube feeding	Never	33	89
	Yes, always (almost always)	0	0
	Yes, when the calf does not suckle	4	11
Maximum time for 90% of calves from birth to first feeding	Less than 2 hours	8	22
	Between 2 and 4 hours	4	11
	Between 4 and 6 hours	15	40
	After 6 hours	7	19
	Suckles the dam	3	8
Amount of colostrum offered at first feeding	≤1L	2	6
	1,1-2L	23	62
	>2L	9	24

	Suckles the dam	3	8
Source of colostrum	Their mother	33	89
	Colostrum bank	1	3
	Suckles the dam	3	8
Is all colostrum used, independent on cow lactation number	Yes	36	97
	No	1	3
Where goes milking 2-4 after calving	To newborn calves	32	86
	To all calves receiving milk	5	14
	Other	0	0
Is colostrum quality checked	No	34	92
	Yes	1	3
	Sometimes	2	5
Confinement the first week after taken from dam	Single pens	3	8
	Pens to and to	4	11
	Pens more than to	30	81
Maximum age gap in calve pen	<4 weeks	15	40
	4-6 weeks	2	6
	>6 weeks	20	54
Bull calves	Lives	20	54
	Slaughter, if no space, at birth.	0	0
	Slaughter, if no space, at 10 days of age.	17	46
	Anothers	0	0

7.2 Clinical scorings and measurements of calves

Parameter	**Score results**	**Proportion %**	**Mean (SD)**	**Median (IQR)**
Nasal discharge	0: 73 1: 1 2: 1	0: 97, 3 1: 1,3 2: 1,3		
Ear- and head tilt	0: 75 1: 0 2: 0	0: 100 1: 0 2:0		
Eye discharge	0: 75 1: 0 2: 0	0: 100 1: 0 2: 0		
Umbilical region	0: 75 1: 0 2: 0	0: 100 1: 0 2: 0		
Joints	0: 75 1: 0 2: 0	0: 100 1: 0 2: 0		
Weight-bearing	0: 75 1: 0	0: 100 1: 0		
Layer of hair	0: 75 1: 0	0: 100 1: 0		
Diarrhoea	0: 54 1: 19 2: 2	0: 72,0 1: 25,3 2: 2,7		
Hair loss	0: 75 1: 0	0: 100 1: 0		
Body condition	0: 68 1: 7 2: 0	0: 90,7 1: 9,3 2: 0		
Soiling	0: 49 1: 19 2: 7	0: 65,3 1: 25,3 2: 9,3		
Coughing	0: 75 1: 0	0: 100 1: 0		
Weight (kg)			32,9 (4.67)	33.4 (5.3)
Heart-girth measurement (cm)			79.7 (2.97)	80 (3.5)
Age (days)			4.2 (1.65)	4 (2)
Rectal temperature			39.0 (0.42)	39 (0.45)

%Brix 1			7.81 (0.72)	7.9 (0.95)
% Brix 2			7.80 (0.73)	7.9 (0.95)
Brix%			7.81 (0.72)	7.9 (0.975)
Gender	Heifer: 34 Bull: 41	Heifer: 45.3 Bull: 54.7	Heifer: 7.78 (0.83) Bull: 7.83 (0.63)	Heifer: 7.6 (1.11) Bull: 7.9 (0.70)