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FACULTY OF HEALTH AND MEDICAL SCIENCE

Effects of two different housing systems on social behaviour, average daily weight gain and health in young calves raised at a conventional Danish dairy farm

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Supervisor: Dorte Bay Lastein, Adjunkt
Submitted on: January 30th 2019

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Title:	Effects of two different housing systems on social behaviour, average daily weight gain and health in young calves raised at a conventional Danish dairy farm
Topic description:	In the modern dairy industry, calves are often housed individually for the first weeks of life, in pens with limited social contact. This is done, even though previous research have shown evidence of calves having decreased capacity to cope with novel environments and unfamiliar calves. In our master thesis we wanted to test if this difference could be found in a Danish conventional herd, since most research have been conducted at research centres. We therefore studied two individual housing systems with different levels of social contact, where the calves were housed for three-four weeks. Hereafter the calves were moved to a novel pen and mixed with unfamiliar calves. Behaviour during mixing was video recorded and analysed using instantaneous scan sampling and continuous recording. Health scores and weights were obtained and used to investigate if housing system influenced health and weight gains of the calves. As a qualitative perspective two semi-structured interview were performed. This was done to give an insight in opinions and observations regarding behaviour of the calves both during individually housing and after mixing in the novel pen. This cross-disciplinary research method is relevant in an evidence-based veterinary advisory situation, where both quantitative and qualitative methods are used to understand an issue of interest, heighten evidence and motivate farmers.
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Preface and acknowledgements

This master thesis was conducted at Department of Veterinary and Animal Sciences at University of Copenhagen from September 2018 to February 2019 and carefully supervised by Dorte Bay Lastein, DVM, Adjunkt. The compilation of this report has been the final part of our education before we graduate as Doctors of Veterinary Medicine.

Our particular subject of this master thesis originated from our personal interests and an ambition to collect our own data. The study was performed at Lykousminde, a conventional dairy farm located in the southern Jutland. This farm was chosen because we wanted to investigate the effects of two different housing systems on social behaviour and performance in young dairy calves in a traditional Danish production setting. Our conclusions are therefore directed to Danish farmers and veterinarians who work with and consults dairy farms in Denmark.

We would like to thank our supervisor, Dorte Bay Lastein, for her invaluable guidance, engagement and cooperation in our project for these past five months.

A special thanks to Mads Fristrup Schou, Liza Rosenbaum, Søren Saxmose Nielsen, and Carsten Kirkeby for their help and guidance in data processing and analysis. Also, a big thank you is given to Gitte Lykou Petersen and Jens Lykou Petersen, for letting us conduct our study at their farm. A special thanks to Stefanie Rasmussen for her cooperation and good management and caretaking of the calves in the project.

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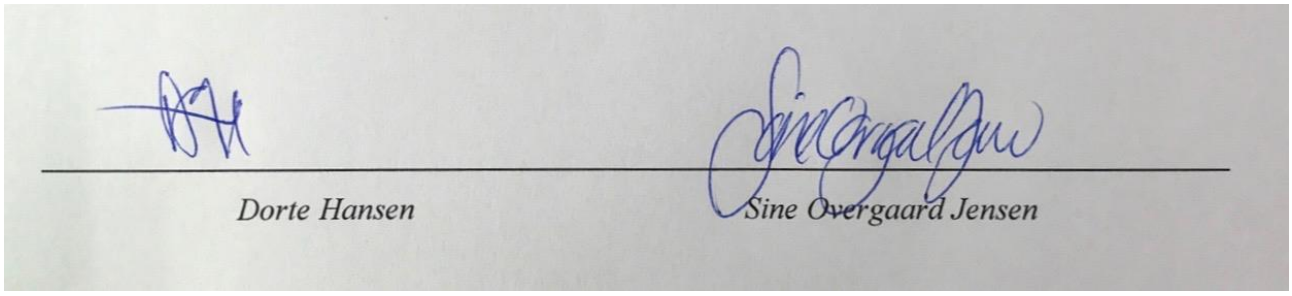
Submitted on January 30th 2019



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Case context

Background and motivation

Throughout our education as veterinarians, we have seen different types of housing and management strategies of calves on dairy farms in Denmark. The strategies are based on raising calves without sickness, with high daily weight gain and with minimum labour and economical cost to the farmer. To achieve all these desired outcomes, the management system does not always fit the calves' natural needs. After having heard a presentation by M. A. G. Von Keyserlingk about calves' cognitive potential, we discovered that this was a subject in which we were both interested. M. A. G. Von Keyserlingk explained how present types of housing systems could delay development of social and cognitive skills, which calves must rely on as future dairy cows. Our interest in this topic was further established as we talked with Gitte Lykou Petersen, one of the owners of Lykousminde, a Danish conventional dairy farm with Holstein-Friesian cows. Gitte Lykou Petersen told us that she could see a difference in the behaviour of their calves, as they are housed individually in two different housing systems before moving to a larger group pen (see *Materials and methods*). She said that the most isolated calves seemed more distressed and fearful compared to the other calves. Therefore, Lykousminde became the obvious choice to carry out a study about calf behaviour and performance between two different housing systems.

For herd veterinarians, the focus have changed from a "diagnose and treat" strategy towards a "health management" strategy, and from "working with animals", towards "working with farmers" (LeBlanc et al., 2006). One challenge in the veterinarian's job, as a consultant to the farmer, is that the farmer and veterinarian might not see the same problems or have different point of views. This situation can lead to frustration and lowered cooperativeness from both farmer and veterinarian which might consequently lead to impaired management. To prevent these situations, qualitative research can be used to understand "why" and "how" the problem has developed and to understand the farmers perspectives to the issue. On the contrary, quantitative research will merely focus on numbers and statistics e.g. prevalence of a specific disease, kilos of lost milk yield etc. Our research was therefore based on the idea of cross-disciplinary research, where both qualitative and quantitative research methods are being used to identify and explain a given issue of interest.

Development of experimental trial design

Our study design followed the calves in their first six to seven weeks of life. Because of the time schedule of our master thesis we decided to only investigate the possible short-term effects of the two different housing systems at Lykousminde. The study design had to fit the already existing housing system of the calves on the farm, and we therefore only aimed to make a small intervention of the housing system. Approximately half of the calves were housed in plywood huts with no contact to other calves other than through a small opening in the side, allowing the calves to see the neighbouring calf (single housed, SH) (see *Materials and methods,*Figure 2). The other half of the calves were housed in plastic huts with a running area in front. These calves were therefore allowed to socially interact over the partition to neighbouring calves (see *Materials and methods*, Figure 3). To make the difference in social contact even bigger between the two housing systems, our plan was to remove the partition which separated the running areas between two neighbouring calves. In this way one pair shared two plastic huts and a common running area in front (pair housed, PH). The calves were assigned to stay in these treatment groups until they were moved to a bigger mixing pen when the youngest calf was three weeks of age. To ensure equal numbers of calves and equal distribution of ages across PH and SH calves, the caretaker had to assign two calves in PH pens, then two calves in SH pens and so forth. In order to quantify potential physiological effects of the two housing systems, calves were weighed and health scored weekly by clinical examination until mixing (see *Materials and methods*). Likewise, medical treatments of the calves, that the main caretaker had performed, were included, to test a possible difference in medical treatments between the two groups.

To study the potential effect of the two housing systems on behaviour, we tested if the PH calves had better social skills and were more competent in handling a stressful situation than SH calves. Therefore, we decided to video record the calves during mixing. The mixing group (MG) would then consist of four calves from each treatment, hence eight calves per MG. We made several pilot studies of the filming procedure and observed behaviour of the calves for several hours after mixing. Finally, we ended up with a procedure of video recording the first 1.5 hours after mixing, starting 30 minutes after the first calf was moved to the pen. To ensure a minimum of disturbance of the calves, we succeeded setting up a system with two video cameras that made it possible for us to leave the calves unattended for the entire filming period.

The video recordings were used for later analysis of each calf with scan sampling at five minutes intervals and continuous recording for selected behaviour types. This observation method and our choices of behaviour types to register, was based on a literature study of animal behaviour in general and previous studies on calf behaviour.

During our study, the calves were taken care of by two different main caretakers. The first main caretaker took care of the calves from the beginning of our study period to mid-October. Hereafter, Stefanie Rasmussen, was hired as main caretaker of the calves. During both periods a third staff member took care of the calves every other weekend.

Due to housing capacity on the farm, it was not possible to assign the calves as we first intended, and the "housing protocol" had to be modified. The first responsible caretaker on the farm was not able to assign the calves in the correct order between the two housing systems, and age of the calves were therefore not evenly distributed between SH and PH pens.
Shortly after the start of the experimental period the farm experienced problems with diarrhoea and pneumonia among both groups of calves. This resulted in increased medical treatments of the calves during the first period of our study, and due to weakness PH calves were housed without full social contact. Since the caretaker was afraid that disease would spread among PH calves, they did not have full social contact during the first three weeks of life. As Stefanie Rasmussen was hired, medical treatments of the calves were markedly reduced. Due to this big difference in treatment and managing of calves we decided to exclude medical treatments performed by caretakers, since they were not representative, and were highly biased. Furthermore, the first caretaker expressed concern that some PH calves were more focused on licking each other rather than drinking their milk during feeding, which caused her to separate them from each other after only a few days together. In order to keep a good relation to the caretaker, we did not want to force her to keep the PH calves as pairs. Altogether, this resulted in a change in the study design of the PH calves, so no intervention was made from the farms' standard housing system. This meant that all calves included in the study were housed individually, but with different levels of social contact to neighbouring calves. In the study paper, the annotation of the treatment groups, are therefore described as "closed-housed" (CH) calves and "open-housed" (OH) calves for SH calves and PH calves respectively.

Development of qualitative perspective

The qualitative perspective of our study consisted of a semi-structured interview of Gitte Lykou Petersen (owner) and Stefanie Rasmussen (caretaker), telling about the possible differences they observe between the calves from the two housing systems. This interview method was chosen because we wanted the answers to be as honest as possible, and because we wanted them to speak freely from their minds. This could not have been achieved by e.g. a multiple choices approach. Their perspectives were important to our study because we wanted to relate the quantitative results to Gitte Lykou Petersen and Stefanie Rasmussen's personal statements about the calves. Furthermore, we wanted to explore if any of our own concerns and expectations on the calves of this specific farm, were the same as theirs. As a veterinarian it is important to understand the farmers opinions of a certain issue, to be able to change the farmers motivational state of mind. For this reason, and as our future work-lives include not only animals but also people, this qualitative part of our study was an important learning process.

We experienced that both Gitte Lykou Petersen and Stefanie Rasmussen were very reflective during the interviews, and that they had a lot on their minds regarding the questions asked. Often their answers lead to multiple other concerns and opinions regarding calves. In the interview situation, this felt as a good progress, but with no previous experience with interviews and processing of these, we found it difficult to analyse their answers afterwards. Therefore, the result of the interviews became more of a depiction of their answers than a direct comparison. Nevertheless, we obtained several important insights in their beliefs and perspectives regarding calves.

Abstract

In the modern dairy industry calves are typically housed individually to achieve higher weight gain, minimize transmission of diseases and lower economic expenses. These housing systems are chosen even though it might compromise the calves' social development. To our knowledge not much is known about the effects of limited social contact of young dairy calves on herd-level. Likewise, low evidence is found for the hypothesis about higher weight gain and increased health status. The present study investigated the effects of different levels of early social contact on behaviour, weight gain and health on a Danish dairy farm. Forty Holstein-Friesian heifer calves were allocated into two different individual housing systems: closed housed (CH) pens with limited contact to other calves (n = 13) or open housed (OH) pens with opportunity for contact with neighbouring calves (n = 27). Calves were weighed and health scored weekly until three-four weeks of age. Calves were moved to a mixing pen (n = 5 groups of 8 calves) at three-four weeks of age. Behaviour was recorded for 1.5 hours at mixing. Registration of selected behaviours were obtained via scan-sampling and continuous recordings.

As a qualitative perspective to the study, a semi-structured interview was performed with one of the owners of the farm and the main caretaker of calves. The interviews were obtained to give an insight in their observations on differences in the calves' behaviours between the two housing systems, as well as their attitudes regarding welfare and care-taking of calves in general. This information was used combines with the quantitative results and our own observations, in order to reach the correct conclusions in the study. This method would have been the most correct approach in an evidence based veterinary advisory situation.

As the study was conducted at herd-level, a liberal p-value was chosen as significance level with $p < 0.15$. During mixing, CH calves laid down more times compared with OH calves ($p < 0.001$) but laid down for fewer seconds per lying ($p = 0.06$). A difference was found for exploring, with CH calves exploring less than OH calves ($p = 0.09$). For unidirectional social behaviour a tendency was found for CH calves expressing this behaviour more than OH calves ($p = 0.17$).

No effect of housing was found on weight gain ($p = 0.93$). A difference in faeces scores between OH and CH calves was found ($p = 0.12$), but no difference in respiration scores ($p=1$)

In conclusion, housing calves with higher level of social contact does not compromise weight gain but could influence health status. These results indicate that socially housed calves might be calmer in a stressful situation compared to isolated calves which are more fearful.

Resumé

I det moderne landbrug opstaldes kalve typisk i individuelle bokse for at opnå højere tilvækst, minimere spredning af sygdomme og sænke økonomiske omkostninger. Denne opstaldningsform er meget udbredt, selvom det potentielt kan forsinke udviklingen af kalvenes sociale færdigheder. Ligeledes findes kun begrænset evidens for en øget tilvækst og højere sundhed. Disse hypoteser undersøges derfor på besætningsniveau i dette studie.

I dette studie blev effekten af opstaldning på adfærd, tilvækst og sundhed undersøgt hos kalve med forskellige grader af social kontakt i de første tre-fire leveuger. Studiet blev udført i en konventionel dansk malkekvægsbesætning. Fyrre Holstein-Friesian kviekalve blev opstaldet enkeltvis i to forskellige opstaldningsbokse: lukkede bokse med begrænset social kontakt (CH kalve; n = 13) eller åbne bokse med mulighed for social kontakt med kalve i tilstødende bokse (OH kalve; n = 27). I denne periode blev kalvene vejet og sundheds-scoret ugentligt. Ved tre-fire leveuger blev kalvene flyttet til en fællesboks, hvor de blev sammenblandet i hold á otte kalve (n = 5 hold). Adfærd ved sammenblanding blev filmet i 1,5 time og evalueret ved brug af momentale og kontinuere registreringer for udvalgte adfærdstyper. Et semistruktureret interview med besætningsejer og kalvepasser dannede grundlag for et kvalitativt perspektiv af studiet. Interviewene omhandlede deres observationer af kalvenes forskellige adfærd mellem de to opstaldningsbokse, samt velfærd og pasning af kalve generelt. Dette blev kombineret med kvantitative resultater og litterær baggrundsviden for at opnå de bedst mulige konklusioner i studiet, som dermed kunne danne grundlag i en evidensbaseret rådgivningssituation.

Da studiet blev udført på besætningsniveau, blev der anvendt en liberal p-værdi med $p < 0.15$ som signifikansniveau. CH kalve lå ned flere gange end OH kalve ($p < 0.001$), men lå ned færre sekunder ad gangen ($p = 0.06$). CH kalve undersøgte miljøet mindre end OH kalve ($p = 0.09$) og havde tendens til at udviste mere envejs social adfærd end OH kalve ($p = 0.17$). Der blev ikke fundet en effekt på tilvækst mellem de to opstaldningstyper ($p = 0.93$). For sundhed var der forskel mellem de to opstaldningstyper i fæces score ($p = 0.12$), men ikke i respirations score ($p = 1$).

Det kan konkluderes at social opstaldning ikke har negative konsekvenser tilvækst, men kan have en effekt på sundhedsstatus. Ydermere indikerer resultaterne at kalve med mulighed for social kontakt i de første leveuger er mindre frygtsomme i et nyt miljø med andre kalve, end kalve opstaldet med begrænset social kontakt.

Introduction

The role of the veterinarian, especially in cattle practice, has changed from a "diagnose and treat" of the individual cow (single-animal-diagnostics) to an approach where focus is on prevention of diseases and management of the entire dairy farm - so called *Herd Health Management* (LeBlanc et al., 2006). Therefore, the veterinarian is merely a consultant to the farmer, in whom the farmer can seek guidance on how to increase the production and optimize health management.
The veterinarian is often presented with multifactorial diseases, where inter-correlated risk factors are of great importance and where the farmers ability or commitment to the problem is very central. It is therefore important for veterinarians to combine both quantitative and qualitative aspects in a veterinary advisory situation. Qualitative research is focused on empirical and inductive research methods, where knowledge is obtained from the given situation, whereas quantitative research is more deductive based on existing theory and knowledge. Often both inductive and deductive methods are useful when a veterinarian is analysing a problem, and in combination the methods will lead to the best evidence-based decision making on a problem related to managing (Lastein et al., 2012). Combining the methods will help understand the theoretical and contextual association between the phenomenon and the willingness of the farmer to implement new management strategies.

A recent control campaign conducted by the Danish Veterinary and Food Administration (Miljø- og Fødevareministeriet, 2018), visited 204 Danish cattle herds (including dairy herds, veal producing herds and herds raising heifers) and a total of 10,985 calves were checked. The campaign concluded that 34.80% of the 204 visited herds failed to fulfil legal requirements (Miljø- og Fødevareministeriet, 2016) concerning the physiological and behavioural needs of the calves. Especially the requirements covering sucking behaviours and individual housing after eight weeks were violated. Focus on health, welfare and management of calves could help increase the standards in the Danish dairy industry. One attempt to heighten the farmers interests in management of calves, came from the interest group Seges, who recently published *Kalveliv* – an informative webpage about feeding, housing, management and health of calves (Seges, 2019). Like the ambitions of the dairy industry are changing, consumer demands are also transforming, especially with products of dairy and meat origin. Consumers ask for higher animal welfare, and a trend towards more

naturalistic production settings is rising (Danmarks Statistik, 2019). A recent survey, *Imaging the ideal dairy farm* (Cardoso et al., 2016), found that animal welfare was the primary issue of interest for the participants, who were not affiliated in the dairy industry. From ethical perspectives, participants expressed that cows should be treated humanely and with respect, and desire for naturalness was often preferred (Cardoso et al., 2016). To meet the consumers' interests Ministry of Environment and Food of Denmark has released an animal welfare label called *Bedre Dyrevelfærd* (Fødevarestyrelsen, 2019). Today there are several kinds of labels concerning animal welfare on the market (Coop A/S, 2019; Danish Crown, 2019; Dyrenes Beskyttelse, 2019). These labels aim to guarantee a higher welfare standard of the animals in their productions. Animal welfare is a complex issue since ethical, cultural and personal opinions might differ between farmers, consumers and veterinarians. These differences might potentially lead to discussions, general reluctance and dejection between the involved actors.

One specific consumer concern regarding the dairy industry are welfare of dairy calves, especially the early separating from dam. Recently, this issue was addressed through a campaign called *12 timer med mor* (12 hours with mom) by the animal rights organization Anima (Anima, 2019). Cattle are social animals which seek companions and form groups in their natural habitat and have long-term hierarchies and social peers whom they prefer (Keeling and Gonyou, 2001). When born in nature the calf will solely interact with the dam the first days of life. It will hide in the vegetation and mostly lie down when the dam is out foraging. Hereafter the calf will begin to interact with other calves in the flock. Play-fights are observed from 2 weeks of age. At 3 weeks of age, the calves will spend most of their time together, even though direct interactions between the calves are still limited (Keeling and Gonyou, 2001).
In the modern production the calf is often separated from the dam 12 hours after calving. Hereafter the calf is placed in an individual pen for the first weeks of life to minimise disease transmission and increase productivity. This potentially prevents the calf from developing social skills in this period, because limited contact with other calves is provided. However, studies have shown that calves that form early social bonds and integrate in a group are more likely to become dominant cows (Broom and Leaver, 1978), show reduced anxiety during restraint (Raussi et al., 2010) and are more successful in obtaining the trough compared to single housed calves (Duve et al., 2012). Chua et al. (2002) investigated social behaviour in calves housed either individually (10 calves) or in pairs (10 pairs of pair-housed calves). In this study group, pair housed calves spent

approximately 2% of their time engaged in social behaviours, stood more and moved more. Individually housed calves lied more and spent more time with their head out of the pen, which is in accordance with other studies (Jensen et al., 1997; Babu et al., 2004). Babu et al. (2004) also found that individually housed calves spent relatively more time idle standing, a finding also confirmed for individually housed calves 34-63 days after birth (Tapki, 2007). Even though time spent engaged in social behaviour is low when housed in pairs (Chua et al., 2002), calves are highly motivated to engage in social behaviour and to gain full contact. Operant conditioning was used to test calves' motivation for two different levels of social contact: Either head-contact or full contact (Holm et al., 2002). The study showed that calves were more motivated to gain full contact, than head-contact, and that full contact was of higher value.

Other studies have also revealed that calves form preferential bonds with each other (Færevik et al., 2006; Raussi et al., 2010; Duve and Jensen, 2011). Færevik et al. (2006) found that calves vocalized more when moved to a novel environment alone or with an unfamiliar calf, compared to a familiar calf. They also explored less when left alone compared to when another calf was present.

Several studies have shown differences between individual and group/pair housed calves during tests, where individually housed calves had more agonistic encounters (Veissier et al., 1994), butted and pushed more (Duve and Jensen, 2011) and were more active when placed in a novel environment (De Paula Vieira et al., 2012). Other studies also showed higher degree of fear (Veissier et al., 1994; Jensen et al., 1997), increased motivation for exploring and lower degree of social sniffing/licking (Jensen et al., 1999; Duve and Jensen, 2011) compared to group housed calves. In contrast, Jensen et al. (1999) found that group housed calves had more play fights, sniffed and mounted other calves more than those individually housed. In a social novelty test, calves housed individually explored less and tended to spend more time standing inactive. Individually housed calves had longer latency to initiate social interaction with an unfamiliar calf, performed less head-head contact, vocalized more and initiated fewer synchronous events of running compared with pair housed calves. However, when social contact had been initiated individually housed calves were more persistent in keeping this relation to the other calf (De Paula Vieira et al., 2012). These results indicate that calves are highly motivated for social contact with peers, even at an early age. Calves raised in individual pens seem to be more reactive and fearful when placed in a novel environment or with unfamiliar calves. Individually housed calves are not as socially confident, as they initiate fewer synchronous running events, perform less head-contact, less sniffing/licking and have longer latency to interact with an unfamiliar calf.

Gaillard et al. (2014) investigated the effect of individual versus social housing on cognitive performance. In a reversal learning task, calves learned to discriminate between white and black by rewarding them with milk when correct colour was chosen by the calf in a Y-maze. The task was then reversed so the previously rewarded colour, was now without reward. Pair housed calves made more correct choices throughout the reversal learning session compared to individually housed calves ($p < 0.05$). During a mixing situation De Paula Vieira et al. (2010) found that individually housed calves had longer latency to start using a new feeder in a novel environment compared with pair housed calves. Pair housed calves also visited the feeder more often and consumed more starter. In another study session by Gaillard et al. (2014), it was found that socially housed calves became less interested in a novel object over repeated sessions, whereas individually housed calves showed no declines in time investigating the object. These findings indicate that calves housed individually may have impaired cognitive skills as they show lower degree of behavioural flexibility and have decreased memory capacity. This can possible influence the calf if these cognitive impairments are long-lasting as they will have difficulties adjusting into the production setting.

Performance of calves are often measured by body weight (BW) and average daily gain (ADG), and evaluated as important measurements for production and health (Gelsinger et al., 2016). A meta-analysis indicated that overall farm management might account for most of the variation in milk yield of first lactation. However, this analysis found a synergistic effect between preweaning intake of milk and dry matter on subsequent milk, fat and protein production in first lactation. Likewise, a positive effect of higher preweaning ADG on first lactation production was found (Gelsinger et al., 2016). Pempek et al. (2016) compared individual and pair housing and found that pair housed calves tended to have higher BW in week seven and eight and had greater final BW. Calves in pairs also consumed more grain (Pempek et al., 2016), which is in line with other studies (Babu et al., 2004; De Paula Vieira et al., 2010; Bernal-Rigoli et al., 2012; Costa et al., 2015). One of these studies also found higher final BW for pair housed calves (Costa et al., 2015). In contrast another study showed no effect on concentrate intake and weight gain, with four different levels of social contact (Jensen and Larsen, 2014). Hepola et al. (2006) showed that group housed calves ruminated more, ate more hay and concentrate compared with individually housed calves. On the other hand, Kung et al. (1997) found that calves housed individually consumed more grain, but ADG and final BW did not differ between treatments. It was argued that decreased accessibility and increased

competition in a group pen could be the cause (Kung et al., 1997). Finally, in a food neophobia test calves housed in complex social groups (cows and calves housed together) decreased latency to eat novel feed and consumed more novel feed compared to individually housed calves (Costa et al., 2014).

These results combined show that pair or group housing of calves does not seem to negatively affect the overall BW or ADG, and in several cases consumption of concentrate increases, which might result in higher weight gain for calves housed with social contact. Several authors have argued that it might be due to social facilitation (De Paula Vieira et al., 2010; Costa et al., 2014, 2016), where calves imitate other calves behaviours.

Diverging results regarding health and performance of calves housed with different levels of social contact, i.e. individually, in pairs or as small groups, have been published. Gulliksen et al. (2009) found that calves housed in group pens from two weeks of age had greater risk of death during the first month of life. However, other studies have shown that group housing of preweaned calves has no adverse effect on performance and health (Kung et al., 1997; Chua et al., 2002). Svensson et al. (2003) classified three subcategories of housing of calves: Individual, small group (3-8 calves) with manual feeding or large group (6-30 calves) with automated milk feeding. Incidence of diarrhoea did not differ between groups but were rated more severe in large groups, whereas no difference was found between individual housed calves or calves housed in the small groups. Incidence was higher for respiratory disease in the large groups, but no difference was found between individual and small groups. This corresponds to another study where different levels of contact between calves were evaluated, and no effect were found in clinical scores, amount of pathogens in faeces or amount of serum antibodies against respiratory pathogens between treatments (Jensen and Larsen, 2014).

Many of the studies discussed so far on health, performance and social behaviour between different housing systems have been performed on research centres. Studies conducted on herd-level are more complex due to other influential factors, that could contribute to observed differences. This study was conducted at a Danish dairy farm, with the aim to test if similar results found at research centres could be found on herd-level. Cross-disciplinary research was conducted to achieve the highest possible level of evidence in this study. Quantitative methodology was used to obtain data for statistical analyses which was combined with a qualitative aspect, in form of a semi-structured interview.

The objectives of this cross-disciplinary study were to investigate if different levels of social contact in housing procedures (1) influenced calf behaviour during a mixing situation with other calves in a novel environment, (2) had an effect on health and performance of calves during their first three and six weeks of life, respectively

Materials and methods

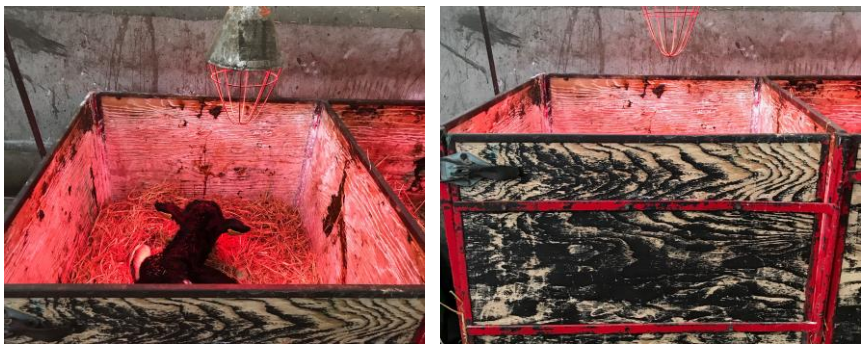
A. Experimental study

The experimental study was conducted from September 2018 to December 2018 at a conventional Danish dairy farm in Southern Jutland. The design was adapted to fit the daily procedures and management system of the calves, already established at the farm.
During the entire study period all calves were taken care of by personnel on the farm and any signs of sickness were treated according to standard operation procedures on the farm (Appendix 1 – Table 1; Appendix 1 – Table 2). During the study period the calves had two different main caretakers; of which Stefanie Rasmussen were caretaker for the final and longest period starting mid-October.

Animals and management

Forty Holstein-Friesian female calves were studied from birth to approximately six weeks of age (WOA). Immediately after birth all calves were separated from their dam and moved to a nursing pen, which consisted of a box measuring 1x1 meter in length filled with straw bedding (Figure 1). Above the nursing pen a heating lamp was placed to help the calves dry. The calves were fed 4 litres of colostrum which was quality checked using a refractometer and heated before feeding to 40^{o}C using the *Coloquick*-system (coloQuick International A/S, 2019). The calves were then assigned to one of two treatments (see *Experimental treatments*) and moved to their respective pen approximately 12 hours post-partum.

Figure 1: Nursing pen. *Two pictures of the nursing pen for new-born calves. Left: Overview. Right: Front view.*



All pens were provided with straw bedding, water and feed buckets. Water and calf starter (Appendix 1 – Figure 1) were available to calves ad libitum. Milk replacer (Appendix 1 – Figure 2) was provided twice a day when calves were housed in individual pens, giving a total of 8 litres per day. At approximately 3 WOA when moved to the mixing pen calves were fed via an automatic milk feeder. The calves were allowed to drink a maximum of 9 litres per day. Ad libitum water, calf starter and baleage were also available. During the entire study period all calves in the project were housed in the same stall building at the farm in the same stall area. The stall also housed dry-cows, a calving area and heifers up to six months of age.

Experimental treatments

Pre-mixing period (0-3 WOA)

The **closed-housed (CH) calves** (n = 13) were housed in pens measuring 217 cm (length) x 108 cm (width) x 123 cm (height) on the inside, making a total space of 2.34 m^2. The sides and backs of the pens were made of plywood, with a roof made up by tin. In each side of the pen, openings were made so calves could have partly contact with the adjacent calves by head to head contact (Figure 2, right). The fronts consisted of vertical metal bars (Figure 2, middle) where the plywood sides made it impossible for the calves to touch and see each other while eating and drinking (Figure 2, left). The outside measure of the sides was 248 cm in length.

Figure 2: Closed-housed (CH) calf pens. *Three pictures of the CH calf pens. Left: One row of pens next to each other in the stall. Middle: Front picture of two pens next to each other, displaying the metal fence. Right: Openings between two adjacent pens (annotated as SH (single housed) calves in case context).*

Open-housed (OH) calves (n = 27) were housed in plastic pens measuring 133 cm (length) x 91 cm (width) with a "running area" in front measuring 135 cm (length) x 109 cm (width), making a total space of 2.68 m^2 per calf (Figure 3). The running area was made up by a partition of vertical metal bars so that the OH calves could see and lick adjacent calves (Figure 3, right).

***Figure 3: Open-housed (OH) calf pens**. Two pictures of the OH calf pens. Left: One row of OH calf pens next to each other in the stall. Right: Two adjacent calves, displaying the front area and metal bar construction. (annotated as PH (pair housed) calves in case context).*

Mixing period (3-6 WOA)

The mixing groups (MGs) (n = 5) were a mix of CH and OH calves (n = 8 calves per MG) (Table 1). The MGs were made according to the calves' birthdates. When the youngest calf was approximately 3 WOA, the calves were moved to the mixing pen measuring 6.70 m x 4.40 m, so that each calf had a space allowance at 3.68 m^2 (Figure 4).

***Figure 4: Mixing pen.** Two pictures of the mixing pen where the mixing groups were housed. Left: Overview of the mixing pen. Right: Front view of the mixing pen, showing the automatic feeding station to the left and the trough and hay rack for solid feed.*

Due to the allocation and capacity on the farm it was not possible to randomize the assignments of calves between treatment groups. Therefore, it was not possible to make a constant proportion of OH and CH calves in the five MGs. The calves were instead allocated to the pens available at the time of birth Only calves with a health score ≤ 2 were moved to the mixing pen and included in the further study. One OH calf was excluded due to compromised health status, which is why MG-4 only consisted of seven calves.

***Table 1. Composition of mixing groups (MGs)**. An overview of the five different MGs composition of open housed (OH) calves and closed housed (CH) calves.*

Mixing group	Number of OH calves	Number of CH calves
1	4	4
2	4	4
3	4	4
4	7	0
5	7	1

Measurements and mixing setup

Pre-mixing period (0-3 WOA)

The first 24 calves born in the study period were weighed for the first time when moved from the nursing pen to either CH or OH pens. Subsequently they were weighed, and health scored once a week on Wednesdays after morning milk feedings. Due to the timeframe it was not possible to weigh and health score the rest of the calves included in the behaviour analysis. Final health scores were obtained on the day of mixing. Final weights were obtained two weeks after mixing. Health scores consisted of a faeces assessment and a clinical examination of the respiratory system combined with rectal temperature. A health score was made respectively for faeces and respiration (Table 2). Health scores were inspired by previous literature (De Paula Vieira et al., 2010; Medrano-Galarza et al., 2018). All weights of the calves were measured on the same scale every time which was calibrated by weighing the same object each week. We alone were in charge of obtaining all weights and health scores, except weighing at birth which was done by the personnel.

Table 2: Health scoring system. *Showing the scoring criteria for faeces and respiratory system. If a score 3 was observed the personnel was noticed and the calf was treated according to the veterinarian consulting the farm (Appendix 1 – Table 1; Appendix 1 – Table 2).*

Score	1	2	3
Faeces	Normal faeces, firmly shaped, yellow or brown colour	Plaques, but not watering diarrhoea and body temperature < 39.5°C	Watery faeces and body temperature ≥ 39.5 °C
Respiration	Absence of nasal discharge and pathological sound	Elevated vesicular respiration or slightly bronchial breathing during auscultation and body temperature < 39.5°C	Elevated or pathological sounds with body temperature ≥ 39.5°C

Mixing period (3-6 WOA)

Before mixing all calves were randomly provided with a collar applied with an individual number. The numbers were randomly assigned to make it possible to distinguish calves from each other on the video recordings, but without knowing the exact individuals. In this way the later evaluations of behaviours were blinded. The calves were moved to the mixing pen in a random order of the collar numbers. The behaviour of the calves was video recorded continuously for 1.5 hours starting 30 minutes after the first calf entered the mixing pen. Video recording was performed with two iPhones (iPhone 5 and iPhone 7 Plus) recording from different angles to ensure a complete overview of the mixing pen. The calves were provided with calf starter, water and milk, which was available in the automatic milk feeder. They were left unattended during the entire recording session.

The recorded behaviours were categorised as posture, feeding, social behaviour and non-social behaviour. Every category was further subdivided in different types (Table 3). All types of behaviours were registered for each calf via scan-sampling every 5th min of the video recordings, except *lying* which was also registered continuously to calculate a total lying seconds. The number of times a calf laid down, was also registered continuously to calculate the average lying time per lying for each calf.

Table 3: Ethogram. *Descriptions of the behaviours registered in the video recordings, showing each category and associated types of behaviours. Sampling is noted as "scan" or "continuous". "Scan" referring to scan sampling with 5-minute intervals. "Continous" referring to the continuous recordings. Recording for lying consisted of lying time in total seconds and number of times a calf laid down.*

Behaviour category	Type (sampling)	Description
Posture	Standing (scan)	Standing still with all four hooves on the ground with lifted or lowered head, and no other apparent activity.
	Standing inactive (scan)	Standing still with all four hooves on the ground with lifted or lowered head, and no other apparent activity for ≥ 10 sec.
	Lying (scan and continuous recording)	Lying on sternum or one side of the body with its head raised, resting on the body or in contact with the ground. The calf might be sleeping, ruminating, manipulating straw with its muzzle or observing the other calves.
Feeding	Feeding (scan)	Muzzle in contact with calf starter in the trough or standing chewing with its head in close contact with the trough.
Social behaviour	Social contact – multidirectional (scan)	Engaged in two-way social interaction with another calf, which can be of both aggressive and non-aggressive character, e.g. sniffing and licking each other, allogrooming, head-head interaction and head-butts.
	Social contact – unidirectional (scan)	Licking or sniffing another calf without getting any attention back from the receiving calf.
	Locomotor play (scan)	Buck: Body is lifted from the ground with forelegs first followed by hindlegs. The buck may be followed by a kick with one or both hind legs in posterior direction. Running: Trotting and galloping are included. At least 4 beats on a forward or circular movement. Jumping: Forelegs lifted from the ground possibly followed by the hind legs.
Non-social behaviour	Exploring (scan)	Sniffing, licking or rubbing against walls, straw and other fixtures of the pen.
	Self-grooming (scan)	Licking its own body or scratching itself with its hoof.
	Walking (scan)	Walking slowly in a symmetrical four beat gait, with no other apparent activity.

Statistical analysis

The data consisted of three subjects – behaviour during mixing, weight until two weeks after mixing and health until mixing – which were analysed separately. All analyses were done in R using R-studio version 1.1.423 (RStudio Team, 2016).

As the study was performed at a conventional farm, a liberal level of significance was chosen instead of the traditional $p < 0.05$. Level of significance was therefore interpreted as $p < 0.15$. This decision was made since many factors could confound results compared to a strictly randomized and blinded research placed at a research-farm (see *Discussion*). Results that were tending to be significant was interpreted as $p < 0.20$.

Behaviour

It was investigated if housing system (OH and CH pens) had an effect on behaviour at mixing. The behaviour types (Table 3) sampled by scans and number of times each calf laid down was not assumed to be normally distributed as they were count data often containing many zeros. A Wilcoxon Rank Sum Test was therefore used to examine if there was any difference between OH and CH calves for each behaviour type.

The total seconds lying down for each calf was assumed to be normally distributed and a Student's t-test was used to calculate the p-value for the difference between housing systems. We also calculated the average number of seconds per lying for each calf, and the same procedure of analysis for total seconds lying down was used. For both analyses, an assessment of the assumption of normal distributed data was done by visual inspection of the data in a qq-plot of the residuals, while the assumption of homogeneity of residuals were assessed from a residuals vs. fitted plot (Appendix 2 – Figure 1; Appendix 2 – Figure 2).

Estimates of the behavioural types were obtained from five different video recordings, each with different individuals, different composition of OH and CH calves and at different dates. We therefore wanted to test if there was a significant effect of filming (i.e. mixing groups) on the behavioural types. For the normally distributed (parametric) behaviour types, linear models were fitted for each behaviour and p-values were obtained with F-tests. For the non-parametric behaviour types a Kruskal-Wallis test was made to obtain p-values. In both parametric and non-parametric analyses behaviours served as response variables and mixing groups as a categorical explanatory variable.

Weight

To examine the effect of the two types of housing systems (OH and CH calf pens) on the average daily weight gain across individuals, we used a general linear mixed model in the R-package *lme4* (Bates et al., 2015). Assumptions of the weight data being normally distributed was confirmed by visual inspection of the data and a qq-plot of the residuals, while the assumption of homogeneity of residuals were assessed from a residuals vs. fitted plot (Appendix 3 – Figure 1; Appendix 3 – Figure 2). The model contained weight as response variable, and age (continuous) and housing system (factorial) as explanatory variables. We also included the interaction between age and housing system, to test if an effect of age differed between the two housing systems. We wanted to allow individual intercepts (birthweight) and slopes (growth rate), as weight measures from the same individual are non-independent. These were included as random effects in the model. To calculate the p-values of the three different terms (age, housing system and interaction) the full model was first compared with a reduced model without the interaction between age and housing system using a likelihood ratio test. The p-value from this test reflects the significance of the interaction, as this is the only term that differs between the two compared models. If a term was non-significant ($p > 0.05$) it was excluded from the model, which was then further reduced by removal of one of the two main effects. P-values for these were then obtained by *likelihood ratio* tests as before.

Health

For the health status (faecal and respiration), each measured by health score (score 1, 2 or 3), we wanted to test if there were differences between housing systems (OH and CH calves). Each of the two datasets (faecal and respiration scores) consisted of a 2x3 table with three response variables (health score 1, 2 or 3, see definitions in (Table 2). Each score was summed for each group, according to Table 7 and Table 8. As some of the frequencies were < 5, we could not use Chi^2 test and instead used the Fisher's Exact test to compare the two housing systems and to estimate the p-value. Our hypotheses were as follows:

H_0: Health status and type of housing are independent

H_A: Health status and type of housing are not independent

Separate tests were performed for each of the two health measures.

B. Semi-structured interview

The qualitative perspective of this master thesis consists of two individually semi-structured interviews (Kvale and Brinkmann, 2015) of Gitte Lykou Petersen and Stefanie Rasmussen. Gitte Lykou Petersen is the one of the owners of the study farm, and highly engaged in calf welfare and -management. Stefanie Rasmussen is the main caretaker of the calves at the study farm. Both interviews were made in Danish and recorded with a Dictaphone app on a Samsung Galaxy 7 and translated to English during analysis. The same interview guide was used for both interviews (Box 1). If any clarifying questions were needed, the primary question was elaborated with questions like *"What do you mean by that?", "What does it mean when you say …?"* and *"Can you give an example?".*

The interview with Gitte Lykou Petersen was performed in November 2018, with location in her private kitchen at the farm. The interview lasted for 39 min. The interview with Stefanie Rasmussen was performed in December 2018, with location in a staff office at the farm. The interview lasted for 35 min.

Box 1: Interview guide. *Display of questions used in the semi-structured interviews.*

Interview guide

Intro:

- Explaining why we want to conduct this interview
- Who are you? Tell us your basics…
- When and why did your interest in calves emerge?

Interview:

- Try to describe the differences you see between the calves raised in brown (CH) and white (OH) huts
    - Why do you think these differences occur?
    - What consequence do you think these differences have for the calves – long-term and short-term?
- What does animal welfare mean to you?
    - What about welfare of calves?
    - Do you think there are welfare issues on this farm regarding calf welfare?
    - What is your view on calf welfare in general in Denmark?
- If you had the opportunity to design your own calf stall, how would it look?
- Do you think Danish farmers are concerned about welfare of calves, and are they trying to improve welfare?
    - What do you think is needed to possibly increase interest and welfare of calves?

Results

A. Experimental study

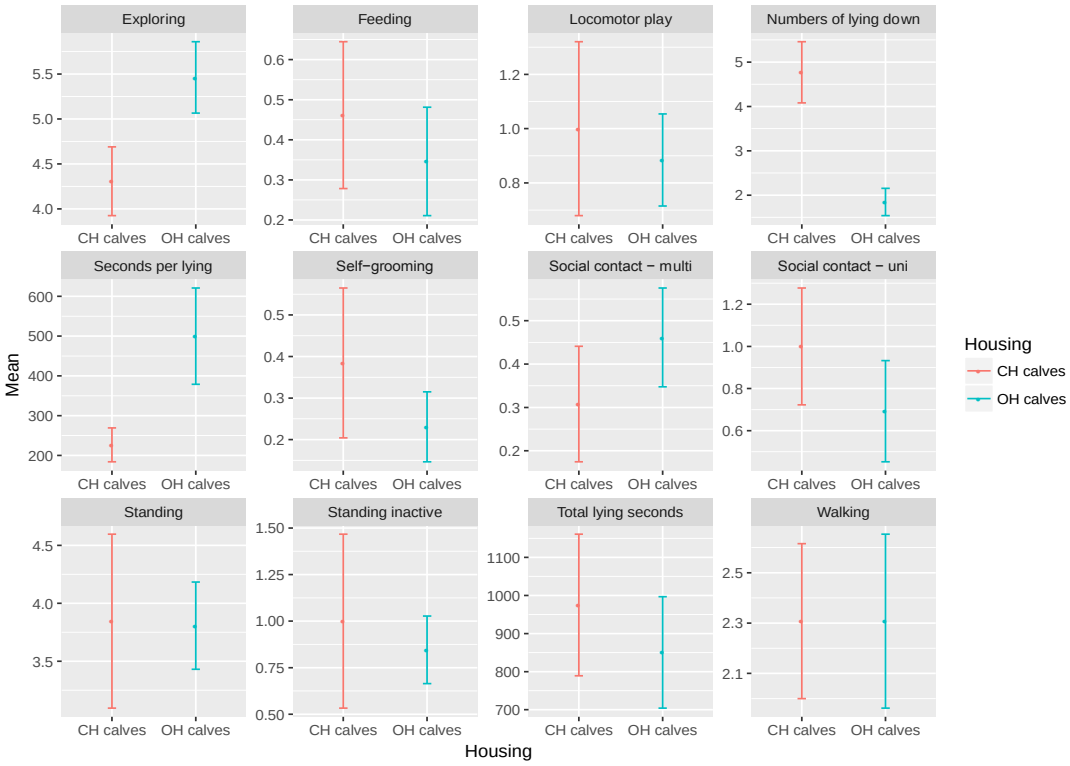
Behaviour

A Pearson's product-moment correlation test between total lying seconds and lying scans showed that these two were highly correlated ($r = 0.96$, $n = 39$, $p < 0.001$), and we therefore focused on one of these, total lying seconds. For the majority of the behavioural types (Table 3), we found no significant differences (liberal p-value, $p < 0.15$, see *Discussion*) between the two housing systems (Table 4). However, the number of times a calf lay down was significantly larger in CH calves (mean ± SD = 4.77 ± 1.38) compared to OH calves (mean ± SD = 1.85 ± 1.57) (Figure 5, Table 4). Results also revealed that seconds per lying was significantly less for CH calves (mean ± SD = 227 ± 154) than OH calves (mean ± SD = 500 ± 617) (Figure 5, Table 4). The high standard deviation of OH calves is due to one individual laying down for more than 2500 seconds. Exploring also showed a significant difference between housing systems, with CH calves being less explorative (mean ± SD = 4.30 ± 1.38) than OH calves (mean ± SD = 5.46 ± 2.02) (Figure 5, Table 4). Finally, for social behaviour we found a tendency towards CH calves showing more unidirectional social behaviour (mean ± SD = 1 ± 1) than the OH calves (mean ± SD = 0.69 ± 1.23) (Figure 5, Table 4).

Table 4: P-values for behaviours between open housed (OH) and closed housed (CH) calves. *Showing the results of analysis of differences between housing systems for each behaviour type. Significant p-values are presented in bold.*

Behaviour:	**Test statistic**	**P-value**
Exploring	W = 112.5	**p = 0.09**
Feeding	W = 190.5	p = 0.43
Locomotor play	W = 171.0	p = 0.96
Lying (scan)	W = 192.5	p = 0.49
Seconds per lying	t (df) = 0.51 (37)	**p = 0.06**
Self-grooming	W = 185.0	p = 0.54
Social contact - multidirectional	W = 147.5	p = 0.46
Social contact – unidirectional	W = 210.5	p = 0.17
Standing	W = 165.0	p = 0.92
Standing inactive	W = 158.5	p = 0.75
Times lying down	W = 292.5	**p < 0.001**
Total lying seconds	t (df) = 0.51 (37)	p = 0.69
Walking	W = 187.5	p = 0.58

***Figure 5: Errorbar plots**. Showing the mean and standard error (SE) for each behaviour type in the two housing systems (OH calves and CH calves).*



Estimates of the behavioural types were obtained from five different video recordings, each with different calves and at different dates. We therefore tested if there was a significant effect of mixing group (MG) on the behavioural types. We found five behavioural types (lying (scan), seconds per lying, social contact – multidirectional, times lying down and total lying seconds) that showed a significant effect of MGs (Table 5). Standing was found tending to show a difference (Table 5)

***Table 5: P-values for behaviours between mixing groups.** Showing the results of analysis of difference between mixing groups for each behaviour type. Significant p-values are presented in bold.*

Behaviour:	**Test statistic**	**P-value**
Exploring	$Chi^2(df) = 5.34\ (4)$	$p = 0.25$
Feeding	$Chi^2(df) = 4.00\ (4)$	$p = 0.41$
Locomotor play	$Chi^2(df) = 1.85\ (4)$	$p = 0.76$
Lying (scan)	$Chi^2(df) = 16.23\ (4)$	**$p < 0.003$**
Seconds per lying	$F\ (df) = 1.93\ (4)$	**$p = 0.13$**
Self-grooming	$Chi^2(df) = 1.69\ (4)$	$p = 0.79$
Social contact - multidirectional	$Chi^2(df) = 22.45\ (4)$	**$p < 0.001$**
Social contact – unidirectional	$Chi^2(df) = 1.90\ (4)$	$p = 0.75$
Standing	$Chi^2(df) = 6.5\ (4)$	$p = 0.16$
Standing inactive	$Chi^2(df) = 3.72\ (4)$	$p = 0.44$
Times lying down	$Chi^2(df) = 9.95\ (4)$	**$p = 0.04$**
Total lying seconds	$F\ (df) = 4.54\ (4)$	**$p < 0.005$**
Walking	$Chi^2(df) = 2.98\ (4)$	$p = 0.56$

Weight

No difference in the effect of age on weight were found between the two housing systems (the interaction term, Table 6), i.e. there was no difference in average daily weight gain between CH calves (mean ± SD = 750 ± 80 grams/day) and OH calves (mean ± SD = 731 ± 94 grams/day) (Figure 6). The OH calves weighed in average 239 g. more at birth than CH calves, although not a significant difference (Table 6). As expected, age had a significant influence on weight (Table 6).

***Figure 6: Average daily weight gain**. A plot of each calf's weight scores from birth to seven weeks of age. The two lines represents an average daily weight gain for the two housing systems with an estimated spread (standard error) of the mean.*

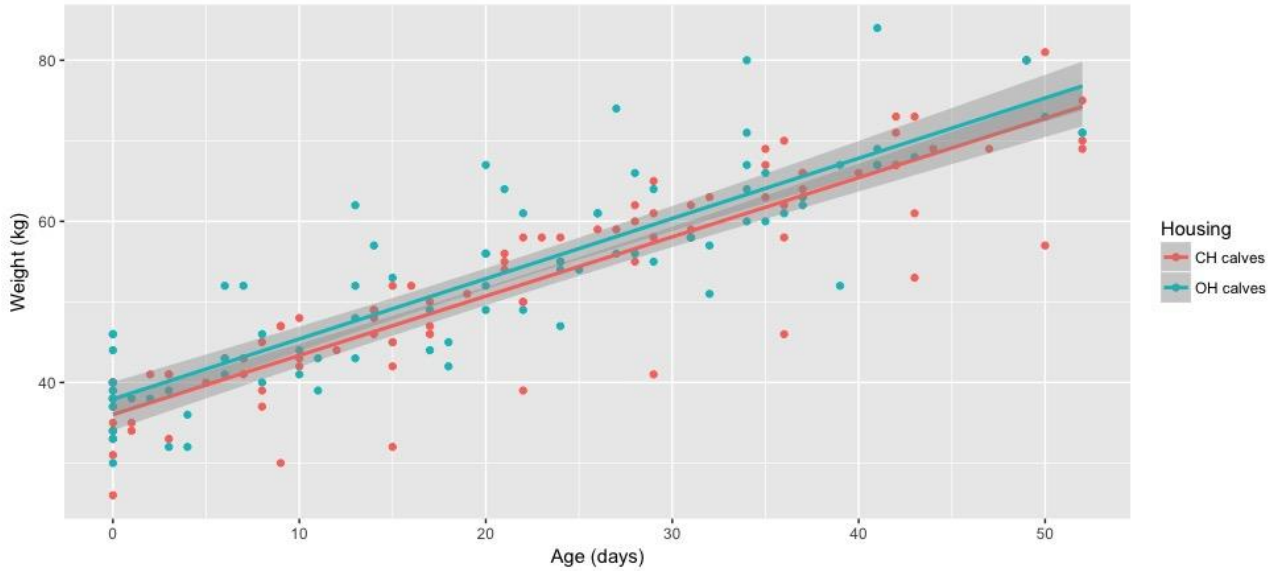


***Table 6: Results from the analysis of weight.** Model coefficients and standard errors (SE) are from the full model before reduction. Statistical test results were obtained by model comparisons using likelihood ratio tests.*

Term	Estimate	SE	$Chi^2_{(df)}$	P-value
Intercept (birthweight)	35.71	1.39	-	-
Age	0.75	0.02	104.80 (1)	< 0.001
Housing system (OH)	2.39	1.97	2.05 (1)	0.15
Age*Housing system (OH)	-0.02	0.04	0.20 (1)	0.65

Health

Faecal health scores showed that only CH calves had faecal score 3 (Figure 7, Table 7). The ones who got a score 3, were calves who already had been given a score 2 previously (Appendix 4 – Figure 1). The CH calves had higher amounts of score 1, than the OH calves, who instead had higher amounts of score 2 (Figure 7, Table 7). Our Fisher's Exact test of the total sum of each faecal score (Table 7) showed a significant difference ($p = 0.12$) between faecal scores in the two housing systems. Therefore, our null-hypothesis could be rejected and H_A concluded to be true: Health status and type of housing are not independent.

Figure 7: Faecal score plot. *A bar chart of faecal scores for CH calves and OH calves respectively. Scores are represented for each week and summed in score 1, 2 and 3. "No score" represent two calves not given scores in week four.*

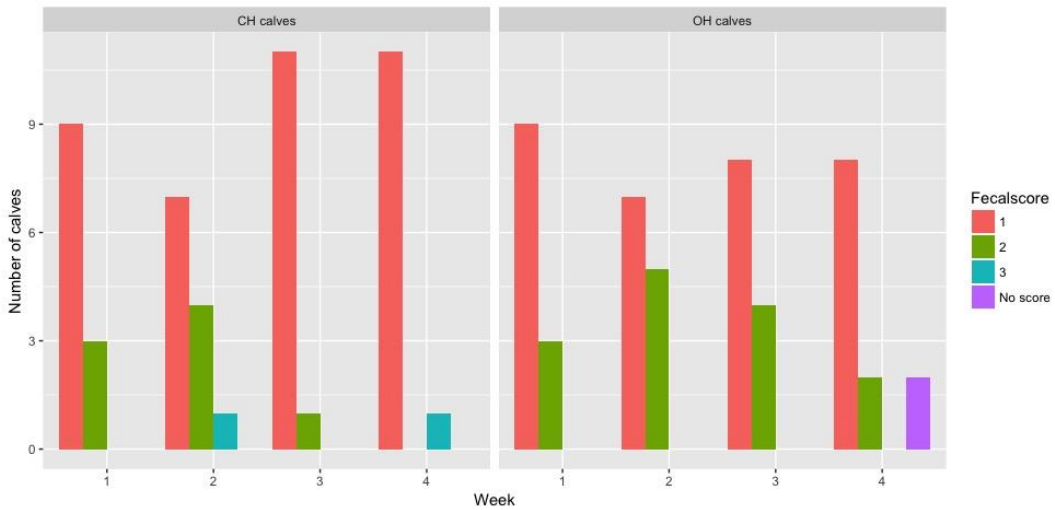


Table 7: Faecal score contingency table*. Shows the total sum of the three different faecal scores from four health checks, for CH and OH calves respectively.*

	Score 1	Score 2	Score 3	Sum
CH calves	38	8	2	48
OH calves	32	14	0	46
Sum	70	22	2	94

Respiration health scores showed that both groups had all three scores and similar distribution of the scores given each week (Figure 8, Table 8). Our Fisher's Exact test of the total sum of each faecal score (Table 8) showed no difference between housing groups (p = 1), and the null-hypothesis could not be rejected. An overview of each calves weekly scores showed that calves given score 3, was only from calves given score 1 the previous week (Appendix 4 – Figure 2).

Figure 8: Respiration score plot*. A bar chart of respiration scores for CH calves and OH calves respectively. Scores are represented for each week and summed in score 1, 2 and 3. "No score" represent two calves not given scores in week four.*

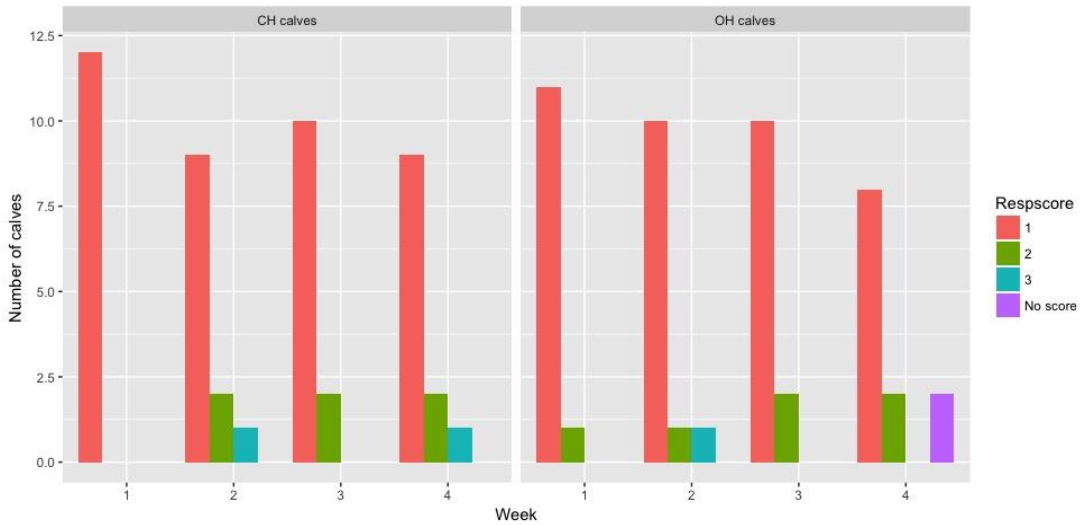


Table 8: Respiration score contingency table*. Shows the total sum of the three different respiration scores from four health checks, for CH calves and OH calves respectively.*

	Score 1	**Score 2**	**Score 3**	**Sum**
CH calves	40	6	2	48
OH calves	39	6	1	46
Sum	79	12	3	94

B. Semi-structured interview

Interview Gitte Lykou Petersen and Stefanie Rasmussen

As a part of the study we wanted to asses a qualitative assessment of calf management and calf welfare on the study farm. This was done by two interviews; one with Gitte Lykou Petersen (owner) and one with Stefanie Rasmussen (caretaker) (see *Materials and methods).* In addition, a more general point of view regarding calf welfare was obtained. This was done to get a better understanding of their opinions regarding calf welfare on the study farm. The interviews were performed in Danish and translated during analysis and reporting. Therefore, the presented answers are not completely identical to the true answers given. Gitte Lykou Petersen was very reflective in her answers which resulted in many different turns and a more indirect answer to many of the planned questions. Stefanie Rasmussen was also reflective, but the interview did not depart much from the interview guide (Box 1). These differences in the interview meant that comparison of their opinions on a question was not always possible. From now on, in this section Gitte Lykou Petersen is referred to as Gitte, and Stefanie Rasmussen as Stefanie.

The first part of the interviews focused on Gitte and Stefanie – who they are, why do they work with calves and why their interest in calves arose (Box 2). This information give insight in their personalities, their experiences and knowledge about calves. Stefanie is very young compared to Gitte who has a long background and experience in farming. This difference may be reflected in different point of views. Furthermore, Gitte is self-taught and has no agricultural education whereas Stefanie is enrolled at agricultural school.

***Box 2**: **Presentation of Gitte and Stefanie**. First part of both interviews with Gitte and Stefanie, explaining their background.*

Interviewer: Can you tell us who you are, what are your basics and from where did your interest in calves emerge?

Gitte: I'm married to a farmer and we have had this farm since 1984, and I've been helping at the farm since 1986 (…). Regarding calves I'm self-taught, and I have never been enrolled at agricultural school. Just as with children, it is self-evident how a calf should have it. It has become a passion of mine (…). The thing about calves is that you must care for them, you must like animals (…). I don't take care of the calves anymore but consult our caretaker of calves when needed.

Stefanie: I'm 17 years old (…), come from a farm with 60 cows in tie stall. I have no previous experience with calves, nor at home (…). I started at Agricultural School because I like animals, and just as much to get out and see if it was something for me (…) and I'm happy about it.

The following section (Box 3) of the interview focused on the differences Gitte and Stefanie had discovered between the calves raised in the two housing systems (see *Materials and methods*). Their opinions included differences seen in behaviour when the calves were housed individually, and the differences seen immediately after moving to a mixing pen (see *Materials and methods*). The two housing systems are called "white" and "brown" huts in the following, which refers to OH and CH calf pens, respectively. These observations from Gitte and Stefanie are important because they were one of the reasons why we wanted to conduct the study at this particular farm. Furthermore, the knowledge about their observations gives some insight in their different point of views. This is relevant, since it is two opinions regarding the same issue. As a veterinarian it is important to obtain more than one person's perspective of a subject, since you might have to use different strategies when trying to motivate people to make a change, as they may have different points of view. For the question asked (Box 3), Gitte focused a lot on the differences at mixing, while Stefanie's observations primarily were while the calves stayed in individual huts. Nevertheless, both Gitte and Stefanie talked about the calves from the brown huts being more observant of the other calves and wilder when moved to the mixing pen. They also both mentioned the reason why calves from the white huts are calmer is because they can see more from their pens, than calves in brown huts.

Box 3: Differences between brown and white huts. *Gitte and Stefanie's observations of the calves raised in the two different housing systems, brown referring to CH calf pens and white referring to OH calf pens.*

Interviewer: Try to describe the differences you see between the calves raised in brown and white huts?

Gitte: We have had the brown huts for years, they only have a little peephole which is mandatory (…). Every time we mixed calves it was obvious to me, that when moved to a group pen with other calves, it seemed (pause) almost as they were afraid of each other (…). They have never seen another calf before, (…) you can tell that "wow, they are jumping a lot", and they act like "wow, there's another calf jumping". It is easy to observe (…). I can see that it gives another kind of calmness for the calves in white huts because they have full vision. They are calmer because they can see. They know what another calf looks like.

Stefanie: The brown calves are more curious than those in white huts, because those in brown huts do not have the same view from their hut as those in white huts, (…) they can see everything

from where they lie (…). The calves in brown huts come out more to see what's happening, because when they lie down, it is a rather closed box.

Interviewer, clarifying question for Stefanie: When moved to the group pen, do you feel that some calves are quicker to learn how to use the automated milk feeder?

Stefanie: You can tell if they have been sick or not, they are slower to learn in general (…). I cannot tell a difference between huts. However it should be that the calves raised in brown huts are more curious, they are also more "wild" when moved to the pen, they are a bit more (pause); I also saw this the last time we moved calves, one of the brown ones, it just keeps running and it never lies down when I'm in the pen (…), it's completely wild and loves the automated milk feeder.

The next question regarded any possible short- or long-term consequences of housing in brown or white huts (Box 4). Stefanie did not believe in any long-term effect of housing system but had strong opinions about the impact that the caretaker has on calves, which is also a very important aspect of managing of calves. It was clear that Gitte had never thought about any possible long-term effects of housing system before and had to reflect a lot upon this question. She ended up, after a long prologue to convince herself, that no long-term effects of housing exist.

Box 4: Consequences of housing systems. *Stefanie and Gitte's perspectives and thoughts about any short- and long-term effects. The extractions primarily include their opinions on long-term effects, as short-term are discussed previously (Box 3.)*

Interviewer: What consequences do you think this difference has to the calves? Possible long-term effect, if any at all?

Stefanie: I think that it is more a question of you who are as a caretaker, the way you interact with the calves from the beginning of their life, already when housed in the nursing pen. The first 24 hours means a lot. I can tell if calves have been petted or just received colostrum (…). They are only housed in huts for 2 weeks and then moved to the automated milk feeder, and I think it is here they start to evolve, and where you can affect them again.

Gitte: I cannot answer that for sure. I don't know (pause). I have never really thought about that.

Interviewer, clarifying question for Gitte: If you were to guess, do you think it could have an effect?

Gitte: No, I don't think so. After a couple of days [in mixing pen] calves seem to be alike. I have observed that (pause). Calves housed in brown hutches have had just as a pleasant life as those raised in white hutches (…). Otherwise half of our calves would turn out to be stressed cows, because half of them are raised in brown hutches, and they are not (…). I am in doubt now. If I were to lie in a dark hut [giggles], I would probably be like "Yes, finally I'm out in the light" when moved. The brown huts are dark for the calves. It's not the right thing.

Stefanie's opinions about how the caretaker influences the calves on daily basis was something she kept referring to in her interview. Gitte also expressed some similar point of views; that particular young people could have trouble becoming a good caretaker of calves (Box 5). Both Stefanie and Gitte expressed how important it is that the caretaker is patient with the calves and avoid rushing things.

***Box 5: About being a good caretaker**. Quotes from Gitte and Stefanie about the importance of acting patiently towards the calves, to be a good caretaker.*

Gitte: (…) but that thing about becoming a good caretaker, it's something you don't easily become. I don't believe that. It has to be a part of them (…). Once we had a young boy (…), every time a tractor came by (pause, stops). Some boys are good at calves (…). You have to be patient with calves, and that's a general problem with men (…), you have to take your time.

Stefanie: It means a lot how you act, even when it's small [the calf]. The calves come to you more often if you have taken your time, and if you don't yell at it or hits it or pushes it. There is some people who does that. It means a lot. You can feel that on all calves, even the big ones (…).

In the following section (Box 6), perspectives were on the social behaviour of calves. Gitte was questioned about her observations of calves raised in white huts expressing social behaviour. Only Gitte was asked this question because of her interest regarding calves' wellbeing and welfare and due to the interview taking a turn towards these subjects. Stefanie was not asked these questions as they were not part of the interview guide (Box 1).

***Box 6: Social contact between calves in white huts.** Extractions of Gitte's observations of social behaviour between calves housed in the white huts. White huts are referring to OH pens.*

Interviewer: How about social contact when calves are raised in the white huts? When do you see, that they are interested in their neighbouring calf?

Gitte: They are interested from the beginning (…) already in the "nursing pen", even though it is closed, the sides are not that high, you can see them licking each other (…). Of course, they would like contact, and if they were housed in the same hut they would lie up against each other (pause) and it would be nice for them.

Interviewer, clarifying question for Gitte: You mentioned that they jump, when another calf jumps. Can you also tell if they want to eat at the same time and that they observe each other?

Gitte: Yes, you can see that (pause) especially in the start they observe each other "What does the other one do?". It stops at some point, when they are more confident (…). You can tell that they observe each other, and if one calf does it, then another calf might do it too. I haven't noticed if

one type [brown or white hut] of calves does it more than the other (…). I think that just the opportunity to touch each other has a tremendous impact (…). If they have not seen another calf before, then it is evident.

The next part of the interviews (Box 7) focused on a more general view on welfare of calves in Denmark. Only Stefanie was asked these questions directly according to the interview guide. The questions were asked to define welfare, and how she would measure it.

Box 7: Stefanie's thoughts about welfare.

Interviewer: What does the term "welfare" mean to you, and how would you define it?

Stefanie: Welfare is a lot of things I guess (…). I think that welfare is when the calf is doing well. You are not in doubt whether a calf is doing good or bad. If, the calf is doing well and it is not overwhelmed. That's welfare to me. By doing well I mean, that the hut is filled with clean bedding, it's well-nourished, it's free form disease and are petted occasionally.

Interviewer, clarifying question to Stefanie: Overwhelmed, what do you mean by that?

Stefanie: We separate dam and calf immediately after parturition (…). You might think that this is stressful for the heifer or cow, but the calf does not notice it, since it is moved to the "nursing pen", where it sleeps and are fed colostrum (…). It is not in pain, it is doing well.

Interviewer, clarifying question for Stefanie: If you should measure welfare in calves, how would you do it?

Stefanie: Weight gain (…). A calf that grows a lot, and gets fat is a good calf.

The next extractions of the interviews (Box 8) focus on how Danish farmers generally manage their calves. Stefanie talked about how the job as a caretaker of calves is considered a boring and easy job by her fellow students. She claims that calves are not as popular as cows and machines at her age, and that most farms shift between employees taking care of the calves during the week.

Box 8: Calf management in Denmark. *Gitte and Stefanie's perspectives on how other farmers manage their calves, and the how important this job in general is considered to be.*

Interviewer, only addressed to Gitte: Do you think that Danish farmers care enough about their calves and their well-being?

Gitte: Welfare of calves is the most important thing at our farm, but not all farmers think that (…). I can be a bit choked when I visit other farms (…), they are very focused on their cows, but

their calf-stall is not as well-managed, and it might be quite dirty (…), but they have no problems with their calves (…). Maybe everything can be too sterile (…). Our calves are pretty fragile (…), we also "push" our calves. We give them large amounts of feed (…) whereas others say that they should only be fed for maintenance (…), and their calves survives too, but they might not have the same weight gain? What is the definition of a "good" calf? It is the one that turns into a good cow (…). Most farmers today are focused on their calves, otherwise they won't get high-performing cows.

Interviewer, question for Stefanie: You are quite young, and may not have seen a lot of different farms, but do you have an opinion regarding calf welfare and general management in Denmark?

Stefanie: (…) calves are just something to get over with, and when I talk with my fellow students they might claim "Oh, it is a crabby job, and it is so boring. They just need to be fed milk and that's it" (…). It is not popular (…), typically you are 4-5 different persons taking care of the calves with one day a week (…). The standard might not be very high when you just want to get over with it (…). I don't think people are very concerned about well-being of calves.

Since Stefanie studied to become a farmer, we interviewed her about how school could address calves, so that it would be more interesting for the students to learn about calves (Box 9). If calf management became a more central role in the education of farmers, maybe some of the young men mentioned in (Box 5) would change their point of views regarding caretaking of calves.

Box 9: Educational aspect of calf management. *Stefanie's thoughts about her school and how they prioritize calf management.*

Interviewer, question addressed to Stefanie: Do you think the school (Agricultural School) could do something to heighten the interest in calves and how important it is?

Stefanie: They could start by teaching us about calves.

Interviewer: So, you don't have any teaching regarding calves while you are at school?

Stefanie: No, we don't. You receive a paper with questions regarding calves and that's it (…). It was kind of an extra assignment, and it gives you a bit of non-serious view on calves. Cows are our main focus and calves are just an extra assignment. At my School they could start by doing that, I don't know what they do at other schools (…). The school is focused on the cow, but we don't learn "how the cow become a cow". It is easy to adjust the robot, but how you got there, it's unknown for some.

Discussion

This study was performed at a conventional farm and we therefore included liberal p-values above 0.05 (Schneider, 2015). We chose to interpret p-values below 0.15 as significant. This decision was made as many factors on the farm could confound the results, compared to a study at a research farm. One confounding factor could be the difference between the two caretakers' daily management of the calves, e.g. hygiene in pens and feeding buckets as well as the caretakers' handling of the calves in general. Another confounding factor is the timeframe of the experimental study. The study period was running for four months, and the calves were raised in a modern stall with limited isolation, which meant the calves were exposed to very different temperatures and levels of draught. These differences could have had an effect on our health scores. Timeframe and management are unlikely to have affected the two housing groups differently, instead the parameters have likely increased the variation between calves within housing group, thereby decreasing our ability to detect differences between housing groups.

The last confounding factor to mentioned regarding the calves, are the possibility that they might be related to each other which could potentially interfere with results due to genetic similarities. This would be the case if some of the dams have been inseminated with the same bull, which could result in some calves being half-sisters. If many half-sister calves were allocated, by chance, in the same housing system, this could affect their results being more homogenic than the calves in the other housing system.

Our choice of using a more liberal level of significance therefore served to decrease the risk of false negatives. However, this also comes with the cost of increased risk of false positives which should be kept in mind. The choice of a liberal level of significance also originates from the goal of using the findings specific to this farm in a potential advisory situation with the owners of the farm. Finding a potential effect with the possibility of being 85% true is of great importance to the farmer.

Behaviour

In the following section we will discuss how the behaviour types were selected, followed by discussions of our results regarding lying, exploring and social behaviour, compared to previous studies. Finally, we discuss our results of differences between mixing groups (MGs) and what problems this could have had to our primary results of behaviours.

The behaviour types (Table 3) used in this study, were inspired by previously published studies (Holm et al., 2002; Rasmussen et al., 2006; Duve et al., 2012) and pilot studies which we performed at the farm before starting our study. It was important that the behaviours were easy to observe from a distance and that the behaviours did not overlap as this would make differentiation between behaviours challenging. The calves also had to express the behaviours on regular basis during the mixing such that a useful amount of data could be obtained. Finally, we chose behaviours we hypothesized would be affected by a difference in housing system. The recording time of 1.5 hours after moving was based on our pilot studies. At this time the calves began to lie down and expressed less locomotor play and exploring.

Closed housed (CH calves) had a higher total lying time compared to open housed (OH calves) although the difference was not significant. However, number of times lying down was significantly different between housing groups, with CH calves lying down more times than OH calves. We also found that CH calves laid down for significantly shorter time per lying than OH calves, which most likely is related to the higher number of times lying down of CH calves Earlier studies have found that calves housed individually have shown higher degree of activity and reactivity (e.g. defecation, backing-off) when released into a novel environment (Raussi et al., 2003; De Paula Vieira et al., 2012). Another study has shown that individually housed calves are more fearful when released into a novel arena or to an unknown conspecific (Jensen et al., 1997). CH calves were housed under a tin roof prior to mixing and experienced another dimension of space around them and have had very limited interactions with other calves prior to mixing. These studies (Jensen et al., 1997; Raussi et al., 2003; De Paula Vieira et al., 2012) could explain why CH calves were disturbed or displaced more easily and therefore did not lie down for as long time per lying as OH calves, who might be calmer in the mixing situation. From our interview with Gitte Lykou Petersen, it was clear that her view on CH calves were that they express more fear in the mixing situation than OH calves. She

described the OH calves as calmer in the mixing situation. These observations from Gitte Lykou Petersen, is in line with the previous mentioned studies.
OH calves explored significantly more compared to CH calves during mixing. Jensen et al. (1999) found that calves housed individually in closed pens explored a novel arena more than calves housed individually in open pens. This finding was explained with a higher motivation to explore, move around and perform locomotion when previously isolated. This contrasts with our study, which showed the opposite. In the study of Jensen et al. (1999) the calves were released alone into a novel arena. In our study the calves were released in groups of eight calves together with familiar and unfamiliar calves in a novel arena. This could have had a big influence in their expression of exploration, as the calves could be more motivated to express other behaviours, such as social behaviour. The OH calves being more explorative in the present study, could be interpreted as these calves being more socially confident than CH calves.

The expression in unidirectional social behaviour between groups tended to be different ($p < 0.20$), with CH calves spending more time sniffing/licking the other calves in the mixing pen. This could be due to higher motivation for exploring new conspecifics and higher interest in engaging in social contact, compared to OH calves, because of CH calves' previous lack of social interactions.
In a previous study, a social novelty test with an unfamiliar calf was performed with individually housed calves and pair housed calves (De Paula Vieira et al., 2012). The test was performed in a novel arena with one test calf together with the unfamiliar calf. Individually housed calves showed fewer head-head contacts and longer latencies to initiate contact to unknown calves but were more persistent in keeping the contact when first established. This could be in line with the present results as CH calves might be more motivated to explore the other calves, instead of exploring the environment. Duve and Jensen (2011) conducted a preference test in a novel arena. Calves housed individually and as pairs, were released one at a time into the arena, where they could choose between a familiar calf and an unfamiliar calf. They found that pair housed calves performed more social licking/sniffing in general, compared to individually housed calves. However, the proportion of time the individually housed calves licked/sniffed the unfamiliar calf (mean ± SEM = 9.88 ± 5.88) was twice the amount than time spent licking/sniffing the familiar calf (mean ± SEM = 5.13 ± 4.07) (Duve and Jensen, 2011). In the present study it was not investigated whether OH calves spent more time or were in closer proximity with formerly neighbouring calves during mixing. It would have been interesting to test if we could find social preferences among calves at such an early age,

in line with the study of Duve and Jensen (2011). It has been found that calves form long-lasting preferential bonds with companions, i.e. they lie closer to familiar calves in large groups, direct more social grooming towards known peers and associate more with calves from the same group (Veissier et al., 1994; Færevik et al., 2006, 2007; Raussi et al., 2010; Duve and Jensen, 2011). Færevik et al. (2006) found that calves vocalized more when separated from their group alone or together with an unfamiliar calf, compared to separation together with a companion. When separated alone the calves also showed higher degree of immobility. In another study it was shown that calves reared together since they were two weeks of age, developed preferential bonds to each other for at least 1.5 years (Raussi et al., 2010). Veissier et al. (1994) found that group-reared animals had higher hierarchical ranks and won most of their agonistic encounters against animals raised in isolation without companions.
Altogether these studies show that calves form preferential bonds at an early age, can differentiate between peers, and that these bonds seem to be long-lasting. Presence of a familiar calf might have a calming effect on calves in stressful situations, like moving to a new pen. Calves reared in isolation or with little social contact seem to achieve lower hierarchical rank and higher degree of displacements and withdrawals. Therefore, it might be beneficial to raise calves in smaller groups even from an early age, to allow them to develop social bonds and eventually to get calmer animals in the herd. It could have been interesting to investigate these possible long-term effects of housing system of the calves in this study. This was unfortunately not possible in this case due to timeframe and expenditure.

Previous studies have shown that pair housed calves only spend 2% of their time on social behaviours during their first 8 weeks of life (Chua et al., 2002), but are more motivated to gain full access to a peer compared to head contact through metal bars (Holm et al., 2002). This suggest that even though calves only spend 2% of their time committed to social behaviours it is still of great importance for them to have full contact with other calves. It has also been found that calves housed individually laid more down (Jensen et al., 1998; Babu et al., 2004), spent more time idle standing (Tapki, 2007) and played less in their home pen (Valníčková et al., 2015). They also spent more time engaged in non-nutritive sucking or licking objects (Tapki, 2007; Pempek et al., 2016), self-grooming and object play (Pempek et al., 2016), were less active and spent more time with their head out of the pen (Chua et al., 2002). Hence, calves seek peers when housed alone and lowered stimuli from other calves may affect calves to become less active since they are deprived social

stimuli. These findings correspond with our interview with Stefanie Rasmussen, who had the impression that the CH calves spent more time observing and looking out of the pen.

It has to be mentioned that comparing studies regarding socials behaviours are very complicated and has to be done with care. This is due to the many different definitions of behaviours used in the studies, and very different study designs. Only few studies can be fully compared, and more replications of previously performed study designs has to be performed, if clear conclusions of effects of housing systems is to be made.

Randomization of distribution of calves between the two housing systems was not possible on the farm, due to the already established management procedures. Therefore, calves were assigned in the pens available at the time of birth, instead of following a strict randomization prior to birth. The major issue of this procedure is that it caused an uneven distribution of OH and CH calves between two of the mixing groups (MGs) (Table 1).
The behaviours lying (scan), seconds per lying, social contact – multidirectional, times lying down and total lying seconds were found to be different between MGs. Behaviours with significant difference between MGs should be interpreted with care when analysing the effect of housing system and may not be as valid and reliable as expected, in terms of comparing the behaviours across the different MGs. Reasons for these differences could be due to the uneven distribution of OH and CH calves in two MGs. This uneven distribution might have skewed results, as calves tend to imitate other calves' behaviours (Costa et al., 2016). Therefore, a MG with very few CH calves could have influenced their behaviours because they imitate the rest of the group. Another explanation to differences between MGs could be disturbance by surroundings under recordings. Examples of such disturbances are the moving of other calves or opening of the side of the stall, so calves were exposed to wind and could see the outside surroundings. It should however, be noted that a significant difference between MGs as analysed here, does not show how many and which of the MGs that differed from each other, instead it shows that at least two of the MGs were different from each other. It was not further investigated which specific MGs were different from the rest. This could have been interesting in terms of the theory of imitation and the non-even distribution of OH- and CH calves. Another approach to investigate the effect of MG could have been through a multivariable analysis of behaviour types. In this case MG and housing systems would serve as

explanatory variables, and the different behaviour types as response variables. Other explanatory variables could also have been tested, e.g. time at birth and health scores.

Weight gain

Calves are often housed individually the first weeks of life, because it is generally believed that individual housing results in higher weight gain, lowered exposure to infectious agents and horizontal spread of disease and decrease in mortality rates.

Our study showed no effect of housing system on the calves' weight gains. All calves gained weight at equal levels, with only age as a significant variable. This finding is consistent with other studies that found no differences in weight gain pre-weaning between individually housed and pair housed calves (Chua et al., 2002; De Paula Vieira et al., 2010). Some studies have found higher final body weight for pair housed calves (Pempek et al., 2016), group housed calves (Bernal-Rigoli et al., 2012) and combined groups (Tapki, 2007). Managing and housing of calves in these studies varied, so comparison of their results should be done with caution, since the dynamics in pair vs. group housing is different.

In the present study starter intake was not measured because of transport distance to the farm, limited time and resources. This information could have provided some insight in feed intake of the calves, and if there were any differences between the two groups. Intake in solid feed, calf starter or dry matter intake have been found to be higher for group housed calves (Babu et al., 2004; Hepola et al., 2006; Tapki, 2007) and pair housed calves (De Paula Vieira et al., 2010; Costa et al., 2015; Jensen et al., 2015; Miller-Cushon and DeVries, 2016).

In the mixing pen, the calves of our study were fed by an automated milk feeder as well as helped to the machine twice a day by the caretaker, unless they had drunk their ration by themselves. This was done to teach them how to drink from a nipple and to ensure that they got their whole milk ration after mixing. However, it would have been interesting to test possible differences in weight gain or feed intake after mixing, to reveal if OH calves would show higher degree of dominance, flexibility or social facilitation to this change, compared to CH calves.

De Paula Vieira et al. (2010) found higher weight gains for pair housed calves in a mixing situation at day 2 and day 3 after mixing, as they started feeding earlier, spent more time at the feeder, consumed more starter and visited the feeder more frequently than individually housed calves. This is in line with Duve et al. (2012), who found that pair housed calves had higher competitive success

of accessing a trough and had longer duration of feeding. Therefore, higher feed intake might be due to social facilitation, social skills, cognitive capacity and dominance for socially housed calves. Bias of weight measurements is primarily if our calibration of the scale did not work as intended or simple typing mistakes of the weights into the data-sheet. We did not obtain birthweights ourselves, as this was done by personnel on the farm when the calves were moved to the nursing pen. There is a possibility that the personnel did not calibrate the scale every time or wrote the wrong weight to the wrong calf.

Health

In the present study a difference in faecal scores between OH and CH calves was found. OH calves had higher amounts of score 2, but no score 3 in comparison to CH calves (Figure 7). Only CH calves were given score 3, but only in 2 cases out of 48 scorings in total (Figure 7). We therefore suspect that the difference found between groups is due to the three levels of faecal scores, and no difference would be found if score 2 and score 3 were combined as one level. In this way our contingency table would have consisted of two response variables (sick and not sick). Nevertheless, the results indicate that OH calves generally have more diarrhoea than CH calves, but do not develop score 3, which require treatment. We suggest faecal pathogens is easy transmitted to the neighbour in OH pens, but this also have an effect on development of the immune system. Our theory is, that the OH calves get smaller doses of pathogens regularly, and therefore develop a better immune response. CH calves are more sensitive as they could suddenly be exposed to the faecal pathogens if management-hygiene is lowered, and therefore require medical treatment. No differences between groups in respiration scores were found. From this result we suggest that the spread of respiration disease is not affected by housing system, which could be due to aerolization of pathogens.

Chua et al. (2002) argued that managing, calf immunity and spread through faecal-oral or aerolization could be more contributing to transmission of diseases than housing itself. This corresponds to our findings and theories. A recent review also found that several managing factors might be of higher importance in transmission of diseases, than individual or group housing itself (Costa et al., 2016). An observational study including 122 Swedish herds with 3081 calves, found that source of colostrum was associated with higher odds ratio for diarrhoea and severe diarrhoea. It also showed that housing in large group pens (6-30 animals) resulted in more cases of severe diarrhoea, whereas no differences were seen between individual housing or housing in small groups

(Svensson et al., 2003). A study performed with different levels of contact between individually housed calves and pair housed calves, found no effect of housing on prevalence of pathogens in faeces or on faecal scores (Jensen and Larsen, 2014). Nonetheless, two experiments performed by Kung et al. (1997) found that group housed calves had fewer days of medication compared to calves housed individually in pens. However, calves housed individually were manually milk fed, whereas group housed calves were fed through automated milk feed, so results might be confounded (Kung et al., 1997).

At the beginning of the study period, the farm experienced problems with both diarrhoea- and pneumonia requiring medical treatments. Our collection of health scores could be biased, as health scores were only obtained on a weekly, and not a daily. Therefore, some calves might have had undetected sickness in between scoring. Since the new caretaker, Stefanie Rasmussen, was hired disease frequencies were lowered, and the overall hygiene and managing of calves was improved. Interpersonal clinical scores could have been different, but this effect was minimized by examining faeces together and clinically examine the same calf, if in doubt with respiration score. Clinical scores could also be biased if CH calves were scored higher than OH calves unconsciously, because being in the CH pen would affect our decision-making. This information bias would generate a systematic error of qualitative measurements. Another possibility of a quantitative information bias is thermometers, which could measure a bit different, but since only temperatures above 39.5°C for score 3 were used, this bias would have had a minor influence.

Conclusions

This study indicates that closed housed (CH) calves are more fearful compared to open housed (OH) calves in a mixing situation, since they explored less and laid down more often and for shorter periods of time. CH calves tended to perform more unidirectional social behaviour, which possibly indicates that they are more motivated for contact with other calves after having been deprived opportunity for social interactions in their first three weeks of life. More research is needed to conclude in what directions (higher or lower expressions) specific behaviours are affected by different housing systems in different situations, and what consequences this might have to the well-being of the calves on herd-level. Studies where the calves are housed in the different housing systems for their first eight weeks of life, would be of great relevance in Denmark, as this is the legal timespan of individual housing.

Higher degree of social contact did not alter weight gain between the two housing systems, during the calves first five weeks of life. Other studies have found that socially housed calves gain more weight than socially deprived calves. Further studies are needed to investigate if this finding is applicable on herd-level.

It was found that health status might be influenced by housing system, with OH calves having more diarrhoea of score 2 than CH calves. No difference between groups in respiration scores was found. It is suggested that faecal diseases are easier transmitted between calves with social access, but that managing of hygiene has a major influence on development of faecal diseases.

Since, Gitte Lykou Petersen was highly motivated in heightening the welfare of calves, our results could be used in consulting her on the possible positive welfare aspects of buying new pens with opportunity for social interactions. We were able to confirm Gitte Lykou Petersens observations of closed housed (CH) calves being more fearful in the mixing situation and we showed they had higher motivation of exploring the other calves than open housed (OH) calves. We would advise her to replace the CH pens to heighten the welfare on the farm, as the calves in OH pens have better opportunity to develop and express their natural social behaviour

Postscript

In this master thesis we chose to conduct our own study on herd-level, because we both were highly motivated in trying to set up a study and collect the data ourselves. In the beginning we thought that it would be relatively easy, since it was a simple study we wanted to conduct. Even though we tried to prepare the staff at the farm by having meetings preceding the study period, we eventually experienced that different levels of motivation can be a real challenge. The owners of the farm were highly engaged in the study, in contrast to the first caretaker of calves, who had a more negative mindset towards the project, especially the fact that we would like the calves to be housed together. In the beginning of the study we tried to house calves together as intended, but this housing procedure was not followed through. Maybe the first caretaker would have been more committed to the project, if we had had a meeting with her alone so we were completely sure that she understood the concept. We had made up a schedule for her to fill out, whenever a calf was born, but she had difficulties filling it out correctly. It might have been beneficial for us to spend some hours together with her in the stall, followed her working procedures and shown her how to fill out the schedule. However, it was a very important learning process for us, since you cannot motivate a person, who is not committed to the issue of your interest. This is very important as a veterinarian, since the employee needs to be motivated herself to change a given problem. You cannot force farmers and their employees to be motivated, but you can try help them to become motivated themselves. It is therefore important to pay attention to the farmers and what they are motivated for and who they are as persons.

References

Anima. 2019. 12 timer med mor, www.12timermedmor.dk. Date cited: 29-01-2019.

Babu, L.K., H.N. Pandey, and A. Sahoo. 2004. Effect of individual versus group rearing on ethological and physiological responses of crossbred calves. Appl. Anim. Behav. Sci. 87:177–191.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48.

Bernal-Rigoli, J.C., J.D. Allen, J.A. Marchello, S.P. Cuneo, S.R. Garcia, G. Xie, L.W. Hall, C.D. Burrows, and G.C. Duff. 2012. Effects of housing and feeding systems on performance of neonatal Holstein bull calves. J. Anim. Sci. 90:2818–2825.

Broom, D.M., and J.. Leaver. 1978. Effects of group-rearing or partial isolation on later social behaviour of calves. Anim. Behav. 26:1255–1263.

Cardoso, C.S., M.J. Hötzel, D.M. Weary, J.A. Robbins, and M.A.G. von Keyserlingk. 2016. Imagining the ideal dairy farm. J. Dairy Sci. 99:1663–1671.

Chua, B., E. Coenen, J. van Delen, and D.M. Weary. 2002. Effects of pair versus individual housing on the behavior and performance of dairy calves. J. Dairy Sci. 85:360–364.

coloQuick International A/S. 2019. www.coloquick.com/how-to-use/. Date cited: 29-01-2019.

Coop A/S. 2019. Dyrevelfærdshjertet, www.dyrevelfærd.coop.dk/dyrevelfaerdshjertet. Date cited: 29-01-2019.

Costa, J.H.C., R.R. Daros, M.A.G. von Keyserlingk, and D.M. Weary. 2014. Complex social housing reduces food neophobia in dairy calves. J. Dairy Sci. 97:7804–7810.

Costa, J.H.C., M.A.G. von Keyserlingk, and D.M. Weary. 2016. Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. J. Dairy Sci. 99:2453–2467.

Costa, J.H.C., R.K. Meagher, M.A.G. von Keyserlingk, and D.M. Weary. 2015. Early pair housing increases solid feed intake and weight gains in dairy calves. J. Dairy Sci. 98:6381–6386.

Danish Crown. 2019. Friland, www.danishcrown.dk/om-os/datterselskaber/friland/. Date cited: 29-01-2019.

Danmarks Statistik. 2019. Indenrigshandel med økologiske fødevarer, www.dst.dk/da/Statistik/emner/erhvervslivet-paa-tvaers/oekologi/indenrigshandel-med-oekologiske-foedevarer. Date cited: 29-01-2019.

Duve, L.R., and M.B. Jensen. 2011. The level of social contact affects social behaviour in pre-weaned dairy calves. Appl. Anim. Behav. Sci. 135:34–43.

Duve, L.R., D.M. Weary, U. Halekoh, and M.B. Jensen. 2012. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. J. Dairy Sci. 95:6571–6581.

Dyrenes Beskyttelse. 2019. Anbefalet af Dyrenes Beskyttelse, www.dyrenesbeskyttelse.dk/anbefalet-af-dyrenes-beskyttelse. Date cited: 29-01-2019.

Færevik, G., I.L. Andersen, M.B. Jensen, and K.E. Bøe. 2007. Increased group size reduces conflicts and strengthens the preference for familiar group mates after regrouping of weaned dairy calves (Bos taurus). Appl. Anim. Behav. Sci. 108:215–228.

Færevik, G., M.B. Jensen, and K.E. Bøe. 2006. Dairy calves social preferences and the significance of a companion animal during separation from the group. Appl. Anim. Behav. Sci. 99:205–221.

Fødevarestyrelsen. 2019. Bedre Dyrevelfærd, www.foedevarestyrelsen.dk/kampagner/Bedre-dyrevelfaerd/Sider/forside.aspx. Date cited: 29-01-2019.

Gaillard, C., R.K. Meagher, M.A.G. Von Keyserlingk, and D.M. Weary. 2014. Social housing improves dairy calves' performance in two cognitive tests. PLoS One 9:1–6.

Gelsinger, S.L., A.J. Heinrichs, and C.M. Jones. 2016. A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. J. Dairy Sci. 99:6206–6214.

Gulliksen, S.M., K.I. Lie, T. Løken, and O. Østerås. 2009. Calf mortality in Norwegian dairy herds. J. Dairy Sci. 92:2782–2795.

Hepola, H., L. Hänninen, P. Pursiainen, V.M. Tuure, L. Syrjälä-Qvist, M. Pyykkönen, and H. Saloniemi. 2006. Feed intake and oral behaviour of dairy calves housed individually or in groups in warm or cold buildings. Livest. Sci. 105:94–104.

Holm, L., M.B. Jensen, and L.L. Jeppesen. 2002. Calves' motivation for access to two different types of social contact measured by operant conditioning. Appl. Anim. Behav. Sci. 79:175–194.

Jensen, M.B., L.R. Duve, and D.M. Weary. 2015. Pair housing and enhanced milk allowance increase play behavior and improve performance in dairy calves. J. Dairy Sci. 98:2568–2575.

Jensen, M.B., and L.E. Larsen. 2014. Effects of level of social contact on dairy calf behavior and health. J. Dairy Sci. 97:5035–5044.

Jensen, M.B., L. Munksgaard, L. Mogensen, and C.C. Krohn. 1999. Effects of housing in different

social environments on open-field and social responses of female dairy calves. Acta Agric. Scand. Sect. A, Anim. Sci. 49:113–120.

Jensen, M.B., K.S. Vestergaard, and C.C. Krohn. 1998. Play behaviour in dairy calves kept in pens: The effect of social contact and space allowance. Appl. Anim. Behav. Sci. 56:97–108.

Jensen, M.B., K.S. Vestergaard, C.C. Krohn, and L. Munksgaard. 1997. Effect of single versus group housing and space allowance on responses of calves during open-field tests. Appl. Anim. Behav. Sci. 54:109–121.

Keeling, L.J., and H.W. Gonyou. 2001. Social behaviour in farm animals. CABI Publ. 113–120.

Kung, L., S. Demarco, L.N. Siebenson, E. Joyner, G.F.W. Haenlein, and R.M. Morris. 1997. An evaluation of two management systems for rearing calves fed milk replacer. J. Dairy Sci. 80:2529–2533.

Kvale, S., and S. Brinkmann. 2015. Interview - Det kvalitative forskningsinterview som håndværk. Hans Reitzels Forl. 3. edition.

Lastein, D.B., M. Vaarst, and C. Enevoldsen. 2012. Herd-specific randomized trials - An approach for effect evaluation in a dairy herd health management program, Manuscript 1. Acta Vet. Scand. 25–76.

LeBlanc, S.J., K.D. Lissemore, D.F. Kelton, T.F. Duffield, and K.E. Leslie. 2006. Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89:1267–1279.

Medrano-Galarza, C., S.J. LeBlanc, A. Jones-Bitton, T.J. DeVries, J. Rushen, A. Marie de Passillé, M.I. Endres, and D.B. Haley. 2018. Associations between management practices and within-pen prevalence of calf diarrhea and respiratory disease on dairy farms using automated milk feeders. J. Dairy Sci. 101:2293–2308.

Miljø- og Fødevareministeriet. 2016. Bekendtgørelse om beskyttelse af kalve, BEK nr 35 af 11/01/2016.

Miljø- og Fødevareministeriet. 2018. Slutrapport for kampagnen fodring og pasning af kalve under 6 måneder. J. nr. 2017-11-60-00186.

Miller-Cushon, E.K., and T.J. DeVries. 2016. Effect of social housing on the development of feeding behavior and social feeding preferences of dairy calves. J. Dairy Sci. 99:1406–1417.

De Paula Vieira, A., M.A.G. von Keyserlingk, and D.M. Weary. 2010. Effects of pair versus single housing on performance and behavior of dairy calves before and after weaning from milk. J. Dairy Sci. 93:3079–3085.

De Paula Vieira, A., A.M. de Passillé, and D.M. Weary. 2012. Effects of the early social

environment on behavioral responses of dairy calves to novel events. J. Dairy Sci. 95:5149–5155.

Pempek, J.A., M.L. Eastridge, S.S. Swartzwelder, K.M. Daniels, and T.T. Yohe. 2016. Housing system may affect behavior and growth performance of Jersey heifer calves. J. Dairy Sci. 99:569–578.

Rasmussen, L., M.B. Jensen, and L.L. Jeppesen. 2006. The effect of age at introduction and number of milk-portions on the behaviour of group housed dairy calves fed by a computer controlled milk feeder. Appl. Anim. Behav. Sci. 100:153–163.

Raussi, S., B.J. Lensink, A. Boissy, M. Pyykkönen, and I. Veissier. 2003. The effect of contact with conspecifics and humans on calves' behaviour and stress responses. Anim. Welf. 12:191–203.

Raussi, S., S. Niskanen, J. Siivonen, L. Hänninen, H. Hepola, L. Jauhiainen, and I. Veissier. 2010. The formation of preferential relationships at early age in cattle. Behav. Processes 84:726–731.

RStudio Team. 2016. RStudio: Integrated development for R. RStudio, Inc., Boston, MA URL www.rstudio.com/. Date cited: 29-01-2019.

Schneider, J.W. 2015. Null hypothesis significance tests. A mix-up of two different theories: the basis for widespread confusion and numerous misinterpretations. Scientometrics 102:411–432.

Seges. 2019. Kalveliv, www.landbrugsinfo.dk/kvaeg/malkekoeer-og-opdraet/smaakalve/sider/kalveliv-forside.aspx. Date cited: 29-01-2019.

Svensson, C., K. Lundborg, U. Emanuelson, and S.O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Prev. Vet. Med. 58:179–197.

Tapki, I. 2007. Effects of individual or combined housing systems on behavioural and growth responses of dairy calves. Acta Agric. Scand. Sect. A, Anim. Sci. 57:55–60.

Valníčková, B., I. Stěhulová, R. Šárová, and M. Špinka. 2015. The effect of age at separation from the dam and presence of social companions on play behavior and weight gain in dairy calves. J. Dairy Sci. 98:5545–5556.

Veissier, I., V. Gesmier, P. Le Neindre, J.Y. Gautier, and G. Bertrand. 1994. The effects of rearing in individual crates on subsequent social behaviour of veal calves. Appl. Anim. Behav. Sci. 41:199–210.

Appendix 1. Materials and methods

Table 1: Standard operating procedures for medical treatments in Danish. CHR: 47529, active per April 2017. Modified from original version handed out by caretaker on January 24th 2019

Diagnose	Symptomer	Kritiske symptomer	Behandling	Støttebehandling
Tarmbetændelse, kalve	Erkendes ved vandig diarré, feber, nedsat æde-og drikkelyst og evt. dehydrering	Ophørt æde/drikkelyst, apati, lammelse	Parofor: 1 skefuld (25g) pr kalv i munden 1xdgl i 3 dage Metacam: 1ml/40 kg kalv, 1 gang SC	Væske, elektrolytter
Lungebetændelse, kalve	Feber, nedsæt æde- og drikkelyst, evt hoste og/eller næseflåd	Ophørt æde/drikkelyst, apati	Florkem:1ml/15kg kalv,im. med 48timers interval Aquacyklin:10%, 1 ml/40 kg kalv 1 gang Metacam: 1ml/40 kg kalv 1 gang SC	-

Table 2: Standard operating procedures for medical treatments in English. CHR: 47529, active per April 2017. Modified from original version handed out by caretaker on January 24th 2019

Diagnosis	Symptoms	Critical symptoms	Treatment	*Support therapy*
Diarrhoea, calves	Recognized by aqueous diarrhoea, fever, decreased eating and drinking and possibly dehydration	Ceased eating and drinking, apathy, paralysis	Parofor: 1 tbsp (25g) orally per calf 1xdaily for 3 days Metacam: 1ml/40 kg calf, 1 time, SC	Fluid Electrolytes
Pneumonia, calves	Fever, decreased eating and drinking, possibly cough and/or nasal	Ceased eating and drinking, apathy	Florkem:1ml/15kg calf, i.m. with 48 H of interval Aquacyklin:10%, 1 ml/40 kg calf 1 time Metacam: 1ml/40 kg calv 1 time SC	-

Figure 1: Calf starter, ValseStart Grøn. *The table of contents of the supplementary feed given to the calves in the study period.*

ValseStart Grøn

Tilskudsfoder til kalve

Produktbeskrivelse:

- Speciel valset byg sikrer høj fordøjelighed, minimal smuld og ekstra lang holdbarhed
- Strukturrig blanding med grønhø sikrer struktur og stor ædelyst
- Sikrer hurtig og skånsom drøvtyggerfunktion
- Tildlig og problemfri fravæning fra mælk
- Afbalanceret forhold mellem let- og mindre letfordøjelig kulhydrat
- Tilsat propionsyre der giver frisk smag

Indholdsgaranti:	
Råprotein	17,8 %
Råfedt	3,6 %
FE pr. 100 kg	97
Vejledende sammensætning:	
Korn	48-50
heraf dampvalset byg	28-30
heraf hel majs	9-10
heraf hel havre	4-5
Sojaskrå, HP	20-22
Roepiller	9-11
Grønhø	4-5
Melasse	4-5
Rapskager / rapsskrå	4-5
Vitaminer / mineraler	3-4
Solsikkeskrå, afskallet	3-4
Citrus	1-2
Lucerne	1-2
Vegetabilsk fedt	0,5-1
Propionsyre	0,2

Beregnet indhold	**pr. kg**	**pr. FE**
Kg foder		1,03
FE	0,97	
Ford. råprotein	140	144
Fedtsyre	32	33
Sukker	72	74
Stivelse	267	275
Cellevægge	189	195
Ca	10,8	11,1
P	4,3	4,4
Mg	2,3	2,4
Na	2,8	2,9
Tilsat:		
Zink	49	50
Kobber	14	14
Mangan	47	48
Jod	0,5	0,5
Cobolt	0,2	0,2
A-vitamin	14.350	14.780
D3-vitamin	1.289	1.328
E-vitamin	92	95
Selen	0,49	0,50
Vombelastningstal	84,5	

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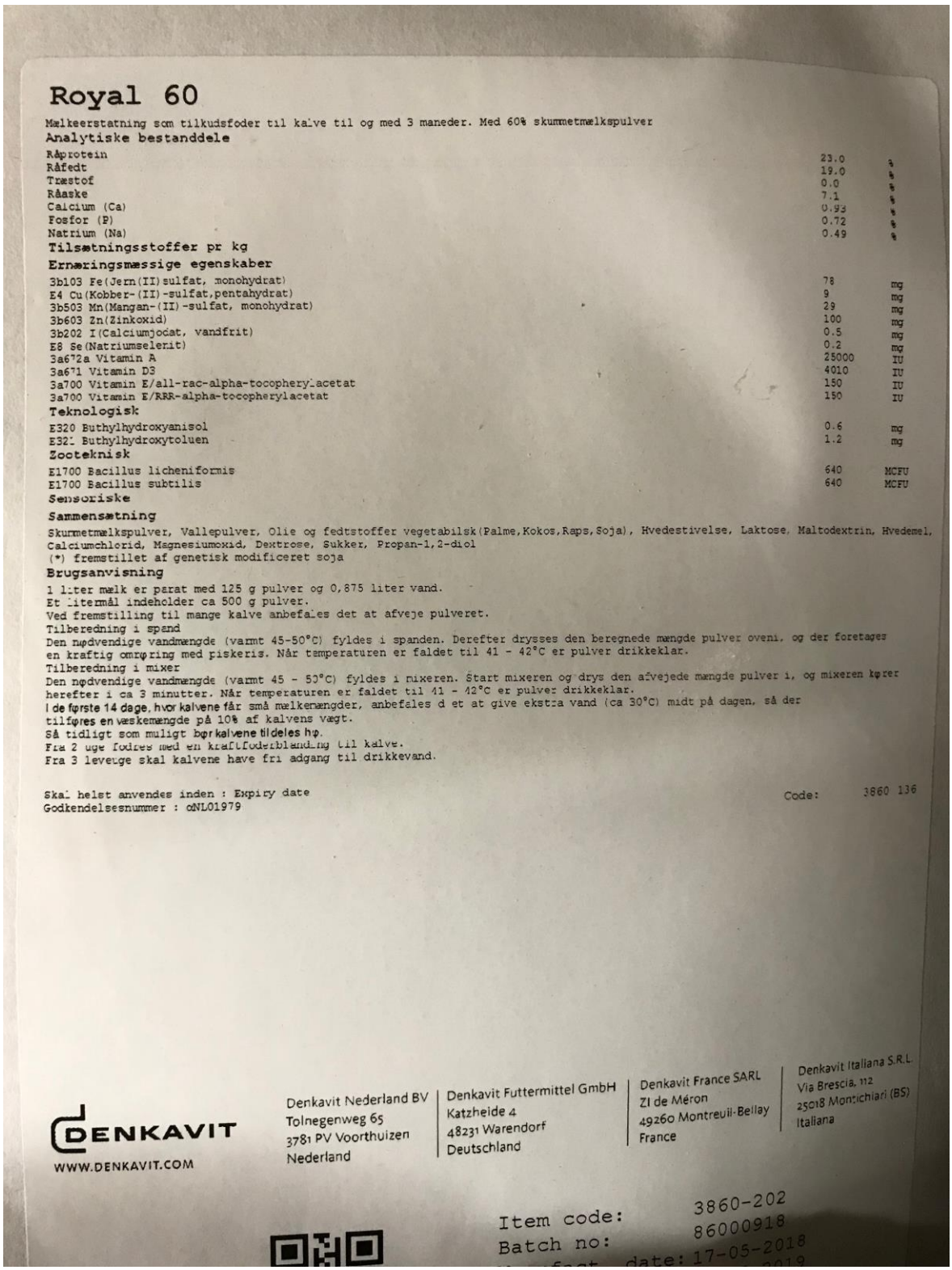
Figure2: Denkavit Royal 60. *The table of contents of the milk replacer given to the calves in the study period.*



Royal 60

Mælkeerstatning som tilkudsfoder til kalve til og med 3 maneder. Med 60% skummetmælkspulver

Analytiske bestanddele

Råprotein	23.0	%
Råfedt	19.0	%
Tørstof	0.0	%
Råaske	7.1	%
Calcium (Ca)	0.93	%
Fosfor (P)	0.72	%
Natrium (Na)	0.49	%

Tilsætningsstoffer pr kg

Ernæringsmæssige egenskaber

3b103 Fe(Jern(II)sulfat, monohydrat)	78	mg
E4 Cu(Kobber-(II)-sulfat,pentahydrat)	9	mg
3b503 Mn(Mangan-(II)-sulfat, monohydrat)	29	mg
3b603 Zn(Zinkoxid)	100	mg
3b202 I(Calciumjodat, vandfrit)	0.5	mg
E8 Se(Natriumselenit)	0.2	mg
3a672a Vitamin A	25000	IU
3a671 Vitamin D3	4010	IU
3a700 Vitamin E/all-rac-alpha-tocopherylacetat	150	IU
3a700 Vitamin E/RRR-alpha-tocopherylacetat	150	IU

Teknologisk

E320 Buthylhydroxyanisol	0.6	mg
E321 Buthylhydroxytoluen	1.2	mg

Zooteknisk

E1700 Bacillus licheniformis	640	MCFU
E1700 Bacillus subtilis	640	MCFU

Sensoriske

Sammensætning

Skurmetmælkspulver, Vallepulver, Olie og fedtstoffer vegetabilsk(Palme,Kokos,Raps,Soja), Hvedestivelse, Laktose, Maltodextrin, Hvedemel, Calciumchlorid, Magnesiumoxid, Dextrose, Sukker, Propan-1,2-diol
(*) fremstillet af genetisk modificeret soja

Brugsanvisning

1 liter mælk er parat med 125 g pulver og 0,875 liter vand.
Et litermål indeholder ca 500 g pulver.
Ved fremstilling til mange kalve anbefales det at afveje pulveret.
Tilberedning i spand
Den nødvendige vandmængde (varmt 45-50°C) fyldes i spanden. Derefter drysses den beregnede mængde pulver oveni, og der foretages en kraftig omrøring med piskeris. Når temperaturen er faldet til 41 - 42°C er pulver drikkeklar.
Tilberedning i mixer
Den nødvendige vandmængde (varmt 45 - 50°C) fyldes i mixeren. Start mixeren og drys den afvejede mængde pulver i, og mixeren kører herefter i ca 3 minutter. Når temperaturen er faldet til 41 - 42°C er pulver drikkeklar.
I de første 14 dage, hvor kalvene får små mælkemængder, anbefales d et at give ekstra vand (ca 30°C) midt på dagen, så der tilføres en væskemængde på 10% af kalvens vægt.
Så tidligt som muligt bør kalvene tildeles hø.
Fra 2 uge fodres med en kraftfoderblanding til kalve.
Fra 3 leverge skal kalvene have fri adgang til drikkevand.

Skal helst anvendes inden : Expiry date
Godkendelsesnummer : oNL01979

Code: 3860 136

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Italiana

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date: 17-05-2018

Appendix 2. Behaviour

Figure 1: Residuals and corresponding qq-plot of total seconds lying down. *Left: Plot of residuals vs fitted values. Right: QQ-plot of standardized residuals.*

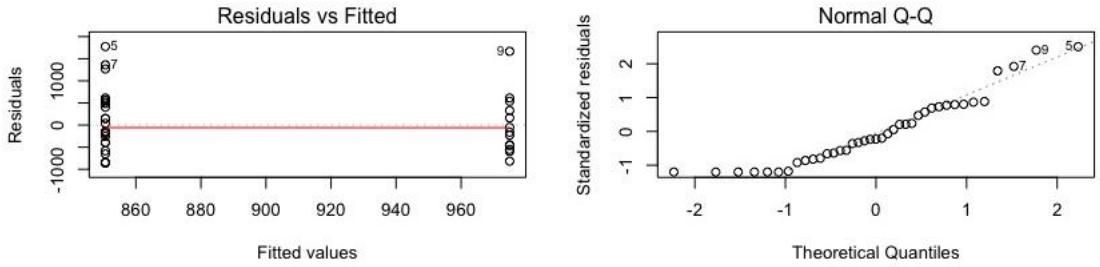
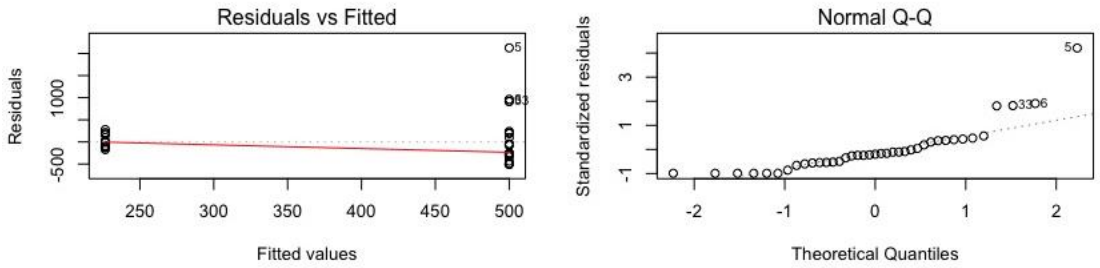


Figure 2: Residuals and corresponding qq-plot of seconds per lying. *Left: Plot of residuals vs fitted values. Right: QQ-plot of standardized residuals.*



Appendix 3. Weight

Figure 1: Individual growth rates. *Raw data plot of every calf's weight curves, dotted lines indicating OH calves and full lines indicating CH calves.*

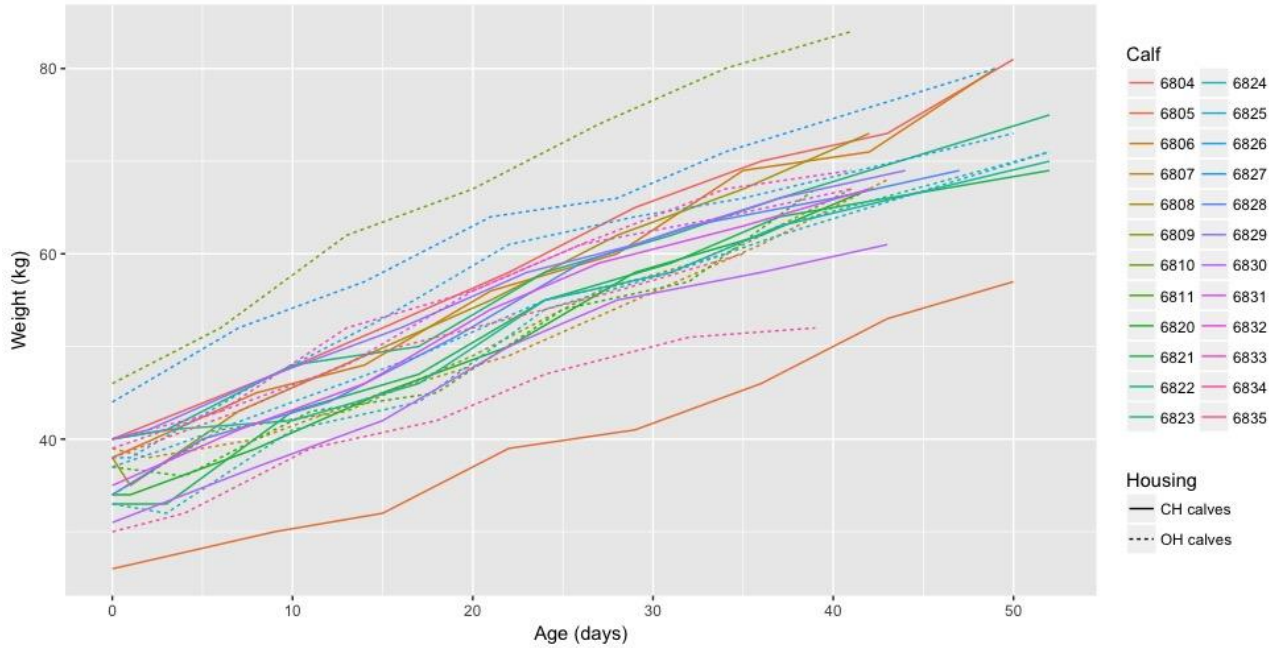
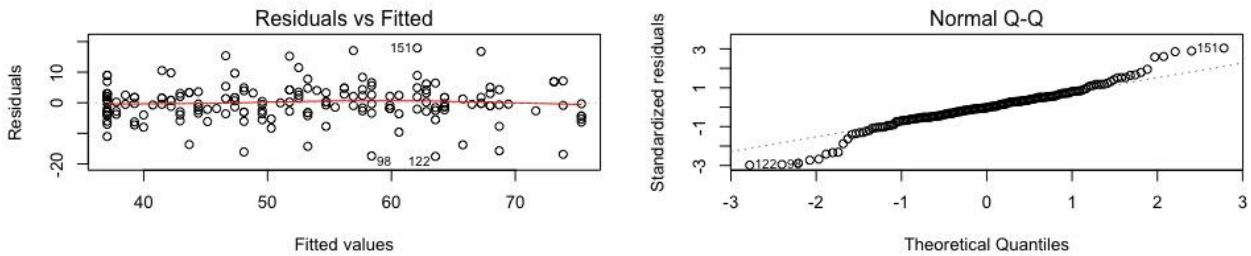


Figure 2: Residuals and corresponding qq-plot of weight data. *Left: Plot of residuals vs fitted values. Right: QQ-plot of standardized residuals.*



Appendix 4. Health

***Figure 1: Individual faecal scores**. An overview of the calves' individual faecal scores once per week in four weeks.*

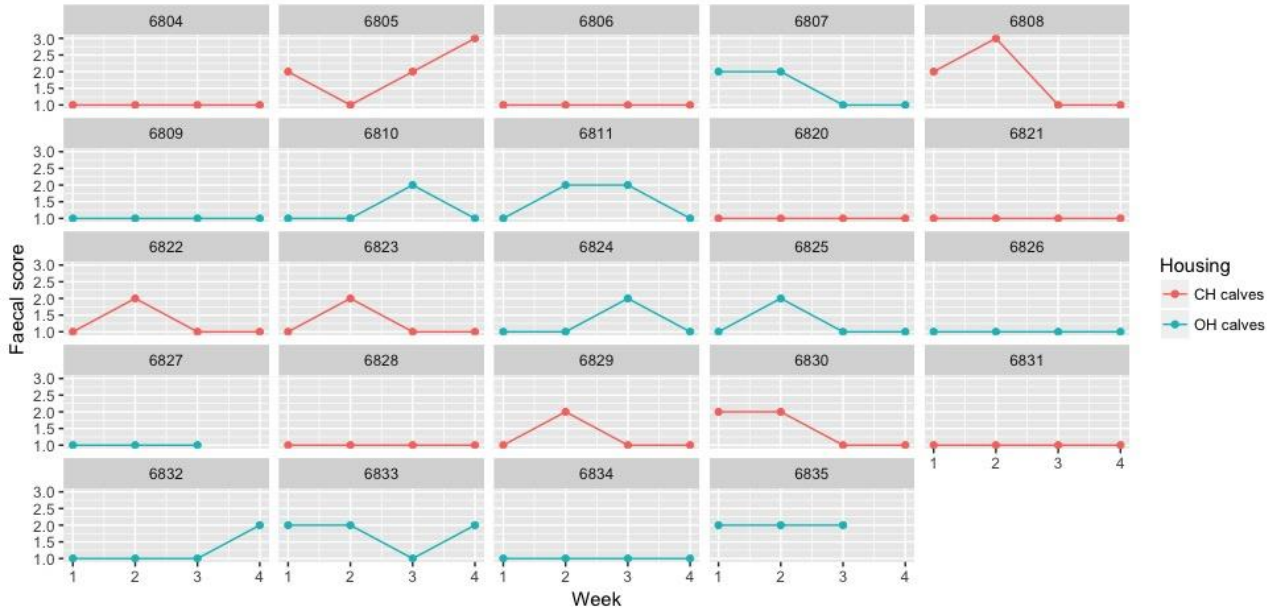


***Figure 2: Individual respiration scores**. An overview of the calves' individual respiration scores once per week in four weeks.*