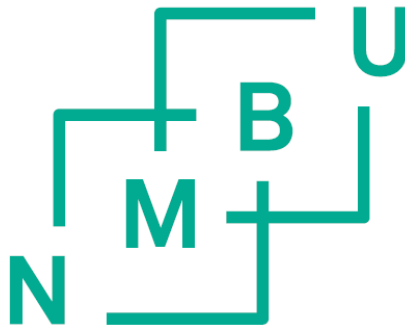# The impact of management on dairy calf welfare

Kristian Ellingsen-Dalskau

Thesis for the degree of Philosophiae Doctor (PhD)

Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences
Department of Production Animal Clinical Sciences

Veterinærinstituttet
Norwegian Veterinary Institute

Oslo, 2015

To my children Ida, Anders and the soon-to-arrive baby without whom
this thesis would have been completed a lot sooner

"Some people talk to animals. Not many listen though. That's the problem."

— A.A. Milne, *Winnie-the-Pooh*

Table of Contents

Preface

This PhD consists of four individual papers which have been written as contributions to three different, previously established projects.

One paper is written as part of Core Organic Aniplan. This project was coordinated by Mette Vaarst (Danish Institute of Agricultural Sciences) and had the overall goal of minimising medicine use in organic dairy herds through animal health and welfare planning.

Another paper is written as part of the Calf Health Project initiated by Vonne Lund (The Norwegian Veterinary Institute), financed by the Norwegian Veterinary Institute. Vonne was responsible for planning the project and collecting the data in collaboration with Petter Stanghov, but sadly passed away before the project was completed.

The two final papers have been conducted as supplements to the Cow-calf project (project number 190424). This project was financed by Foundation for Research Levy on Agricultural Products (FFL) and Agricultural Agreement Research Fund (JA) through the Research Council of Norway. The project aim was to develop robust and economically viable models for cow-calf suckling in organic dairy systems, fulfilling high standards for animal health, welfare and ethics.

Acknowledgements

you, Lina, for being my wife and best friend, for your never-ending support, encouragement, kindness and love. This would not have been possible without you.

Oslo, Norway, October 2015

Kristian Ellingsen-Dalskau

List of abbreviations

HAR   Human-animal relationship

NCHRS   Norwegian Cattle Health Recording System

QBA   Qualitative Behaviour Assessment

SEM   Structural equation modelling

RSP   Response surface pathway

List of papers

The thesis is based on the following papers, referred to by their Roman numerals.

Paper I

Ellingsen, K., Mejdell, C. M., Hansen, B., Grøndahl, A. M., Henriksen, B. I. F., & Vaarst, M.

Veterinarians' and agricultural advisors' perception of calf health and welfare in organic dairy production in Norway

Organic Agriculture, 2012; 2(1), p. 67-77.

Paper II

Ellingsen, K., Coleman, G. J., Lund, V., & Mejdell, C. M.

Using Qualitative Behaviour Assessment to explore the link between stockperson behaviour and dairy calf behaviour

Applied Animal Behaviour Science, 2014; 153, p. 10-17.

Paper III

Johnsen, Julie F., **Ellingsen, K**., Grøndahl, A. M., Bøe, K., Lidfors, L., Mejdell, C. M.

The effect of physical contact between dairy cows and calves during separation on their post-separation behavioural response

Applied Animal Behaviour Science, 2015; 166, p. 11-19.

Paper IV

Ellingsen, K., Mejdell, C. M., Ottesen, N., Larsen, S., & Grøndahl, A. M.

The effect of large milk meals on digestive physiology and behaviour in dairy calves

2nd submission to Physiology & Behavior.

Summary

No universal definition of animal welfare exists. However, an animal allowed expression of natural, innate behaviours, showing good biological functioning and having a positive affective state is generally viewed as having a high level of welfare. Animal welfare challenges exist across all countries and species. This thesis is focused on dairy calves and the impact that management has on their level of welfare.

Organic production has several prerequisites which should allow for better animal welfare than conventional production. The organic legislation generally has higher levels of minimal standards and the organic philosophy has a strong emphasis on the natural behaviour approach to animal welfare. Veterinarians have traditionally focused on the biological functioning approach to animal welfare and have been criticized for being sceptical towards organic farming. To investigate views on calf health and welfare in organic dairy production, and potential differing opinions between veterinarians and agricultural advisors, we sent out a questionnaire to 400 large animal practitioners and 400 agricultural advisors. A total of 207 veterinarians (52%) and 215 agricultural advisors (54%) responded. The questionnaire contained questions related to calf health and welfare in organic dairy production in Norway, as well as some questions comparing organic production with conventional. Results showed that low calf mortality and adequate treatment of ill or injured animals were rated most favourably for calves in organic dairy production. Body condition and growth received the least favourable scores. The calf staying with its mother after birth was seen as the most important welfare advantage, while poor feed quality was seen as the most important welfare challenge. Veterinarians and agricultural advisors generally considered health and welfare to be equal for calves in organic and conventional dairy farming systems in Norway. The findings suggest that management has a greater impact on calf welfare than production system.

A major part of management is the handling of animals during routine procedures like moving or milking. The way stockpeople handle their animals has a great impact on the human-animal relationship and hence the level of animal welfare. To study the effect of handling style on animal behaviour and welfare, we applied Qualitative Behaviour Assessment. Qualitative Behaviour Assessment is a method in which an observer scores a list of descriptors, e.g. aggressive, sociable, content, tense, and happy, from minimum to maximum based on his/her observed behaviours on a farm. One list of descriptors was

completed for the calves, another list for the stockpeople. Principal component analysis classified calf behaviour along two axes labelled positive/negative mood and high/low arousal. The same method on stockperson behaviour revealed four distinct handling styles called calm/patient, positive interactions, dominating/aggressive, and insecure/nervous. While the two former are viewed as positive, the two latter are viewed as negative. Using structural equation modelling, it was found that stockpeople with positive handling styles have calves with higher levels of positive mood and vice versa. These results show the direct link between human and animal behaviour and emphasise the importance of good stockmanship and self-awareness in contact with animals to ensure good animal welfare.

Although a positive human-animal relationship is established, many management procedures are perceived as negative by the animals. One such procedure is the separation of cow and calf. In conventional dairy production the calf is usually separated from the cow immediately after birth, while in organic dairy production separation occurs after three days in Norway and one day in Denmark and Sweden. Separation of a bonded cow-calf pair will inevitably be stressful to the animals. Research has therefore been aimed at designing alternative separation methods to reduce post-separation stress, especially for beef cattle. One alternative separation method is fence-line separation, which allows limited physical contact between the cow and calf after separation through and over a fence. Our results showed that fence-line separated calves performed less high-pitched (open mouthed) vocalizations compared to calves separated by a solid wall. Fence-line separated calves also showed less alert behaviour like gazing and directing ears, head and neck in the direction of the cow. For the cows no differences were discovered. These results suggest that physical contact with the mother after separation can reduce behavioural stress responses in the calf.

Early separation of cow and calf prevents suckling and milk needs to be artificially administered to the calves. It is commonly believed that calves can only be fed 2-3 litres of milk per meal. If higher amounts are given, milk can supposedly enter the rumen causing indigestion, diarrhoea and reduced growth. Unable to find the scientific basis for this belief, we wanted to challenge it. Six Norwegian Red calves at around three weeks of age were, on three test days, given unrestricted portions of warm, whole milk through a teat bottle with a relatively small opening. The milk had a contrast agent, barium sulphate ($BaSO_4$), added to it and abdominal radiographs were taken to see if the milk stayed in the abomasum or entered the rumen. The calves drank high amounts of milk, the highest reaching 6.8 litres (13.2% of body weight) in one meal. The radiographs showed that the abomasum has great capacity for expansion. Regardless of intake, no milk in the rumen or abdominal pain or discomfort was

observed in any of the calves. These results indicate that farmers can increase the milk meal sizes for their calves given that the warm whole milk is given through a teat with a relatively small opening.

Through different approaches and methods I have attempted to shed light on the impact of management on calf welfare. Hopefully this knowledge will make a positive contribution to dairy calf keeping nationally and internationally.

Sammendrag

Selv om det ikke finnes en universell definisjon av dyrevelferd, blir dyr som får utøve naturlig, medfødt atferd, som har god biologisk funksjon og som har en positiv mental tilstand som regel ansett for å ha et høyt velferdsnivå. Dyrevelferdsutfordringer finnes i alle land og blant alle arter. Denne avhandlingen tar for seg kalv i melkeproduksjonen og hvilken betydning driftsopplegg og stell (management) har for dyrevelferdsnivået.

Økologisk produksjon har et regelverk som setter høyere minimumsstandarder på dyrevelferdsområdet enn konvensjonell lovgivning og en filosofi med stort fokus på at dyrene skal få utløp for sin naturlige atferd. Veterinærer har tradisjonelt hatt et ensidig fokus på god helse som grunnlag for god dyrevelferd og har ofte blitt kritisert for å være for negative til økologisk produksjon. For å undersøke synet på kalvehelse og -velferd i økologisk melkeproduksjon, og potensielt ulike vurderinger mellom veterinærer og rådgivere, sendte vi ut en spørreundersøkelse til 400 stordyrpraktikere og 400 rådgivere for økologiske produsenter i Norge. Totalt svarte 207 veterinærer (52 %) og 215 rådgivere (54 %). Undersøkelsen inneholdt spørsmål om kalvehelse og velferd i økologisk produksjon, samt noen spørsmål som sammenliknet økologisk produksjon med konvensjonell. Resultatene viste at lav dødelighet og behandling av syke og skadde dyr ble rangert høyest av forhold som er viktig for å sikre god kalvehelse i økologisk melkeproduksjon. Kroppshold og tilvekst fikk lavest rangering. At kalven fikk gå med kua etter fødsel ble sett på som den største velferdsfordelen i økologisk drift, mens dårlig fôrkvalitet ble sett på som den største utfordringen. Veterinærene og rådgiverne vurderte i stor grad helse og velferd som lik for kalver i økologisk og konvensjonell produksjon i Norge. Disse resultatene tyder at gårdbrukerens prioriteringer og dyrestell har større betydning for kalvevelferden enn selve produksjonssystemet (økologisk/ikke-økologisk).

En stor del av den daglige dyrehåndteringen består av rutinemessige prosedyrer som for eksempel flytting eller melking. Måten røkteren behandler dyrene sine på har stor innvirkning på dyr-menneske-forholdet og dermed nivået av dyrevelferd. For å undersøke innvirkningen av håndteringsstil på dyreatferd og -velferd, benyttet vi kvalitativ atferdsvurdering (QBA). Kvalitativ atferdsvurdering er en metode hvor en observatør fyller ut en liste med beskrivende ord, for eksempel aggressiv, sosial, tilfreds, anspent og glad. Hvert ord gis en score fra minimum til maksimum basert på hvor godt ordet karakteriserer det som ble observert. Én slik liste ble fylt ut for kalvene og en annen for røkteren på hver gård.

Prinsipal komponentanalyse klassifiserte disse beskrivende ordene langs to akser kalt god/dårlig sinnsstemning og høy/lav aktivitet. Den samme metoden på røkteren avdekket fire ulike håndteringsstiler kalt rolig/tålmodig, positive interaksjoner, dominerende/aggressiv og usikker/nervøs. De to første håndteringsstilene er sett på som positive, mens de to siste er sett på som negative. Ved å bruke *structural equation modelling* ble det funnet at røktere med en positiv håndteringsstil hadde kalver med bedre sinnsstemning og vice versa. Disse resultatene viser en direkte sammenheng mellom menneske- og dyreatferd, og fremhever viktigheten av gode røkteregenskaper og bevissthet om egen atferd i kontakt med dyr for å sikre god dyrevelferd.

Selv om et positivt dyr-menneske-forhold er etablert, blir mange av management-rutinene oppfattet som negative av dyrene. En slik rutine er separasjon av ku og kalv. I konvensjonell melkeproduksjon blir kalven som regel skilt fra mora umiddelbart etter fødsel, mens i økologisk melkeproduksjon skjer separasjonen etter minimum tre dager i Norge og én dag i Danmark og Sverige. Separasjon av dyr som har etablert et bånd vil utvilsomt være stressende for dyrene. Forskning har derfor vært viet til å utvikle alternative separasjonsmetoder for å redusere stressnivået, spesielt for kjøttfe. En slik alternativ separasjonsmetode er *fence-line*-separasjon som tillater begrenset fysisk kontakt mellom ku og kalv gjennom og over et gjerde etter separasjon. Våre resultater viste at kalver separert med *fence-line* utførte mindre høyfrekvent vokalisering (med åpen munn) sammenliknet med kalver separert med en tett vegg. *Fence-line*-separerte kalver uttrykte også mindre alert atferd som stirring og retting av ører og hode i kuas retning. For kuene ble det ikke funnet noen forskjeller. Disse resultatene indikerer at fysisk kontakt med moren etter separasjon kan redusere stressnivået hos kalven.

Tidlig separasjon av ku og kalv betyr at melk må tildeles kalvene på andre måter enn ved diing. Det er en vanlig oppfattelse at kalver ikke skal få mer enn 2-3 liter melk per måltid. Hvis større volumer gis, frykter man at melk kan komme inn i vomma og føre til fordøyelsesbesvær, diaré og redusert vekst. Ettersom vi ikke klarte å finne et vitenskapelig grunnlag for denne oppfattelsen, ønsket vi å utfordre den. Seks NRF-kalver, rundt tre uker gamle, fikk på tre testdager tilgang til større porsjoner kroppsvarm helmelk gjennom en smokkeflaske med en relativt liten åpning. Melken var tilsatt bariumsulfat ($BaSO_4$), og det ble tatt røntgenbilder av mageregionen for å se om melken holdt seg i løpen eller havnet i vomma. Kalvene drakk store mengder melk, helt opp til 6,8 liter (13,2 % av kroppsvekten) i ett måltid. Røntgenbildene viste at løpen har stor kapasitet for utvidelse. Uavhengig av inntak, ble det ikke observert melk i vomma eller tegn på magesmerter hos noen av kalvene. Disse

resultatene tyder på at bønder kan øke porsjonsstørrelsen til kalvene forutsatt at det gis varm helmelk gjennom en smokk med en relativt liten åpning.

Gjennom forskjellige tilnærminger og metoder har jeg forsøkt å belyse betydningen av driftsopplegg og stell for kalvevelferden. Forhåpentligvis vil denne kunnskapen være et positivt bidrag til kalvehold nasjonalt og internasjonalt.

Introduction

Animal welfare

The status of animals

People have kept animals for thousands of years. For almost equally long, concern for animal wellbeing has been a topic of attention, often linked to religion. For instance, Hindu and Buddhist scriptures are interpreted as encouraging vegetarianism for ethical reasons (Srivastava, 2007). Christianity also promotes animal welfare, e.g. as stated in the Proverbs 12:10: "The righteous care for the needs of their animals." However, most modern Christians believe in the "dominion perspective" stating that human life has greater value than animal life and that nature exists only for serving the needs and interests of man (Srivastava, 2007).

The moral status of animals was heavily debated among the ancient Greeks. Pythagoras (c. 570– c. 495 BC), a philosopher and mathematician advocated respect for animals. He believed that humans and animals possessed the same kind of soul, making us one with the animals (Phelps, 2007). Opposing these ideas, Aristotle (384–322 BC) claimed that animals had no interests of their own, thereby ranking them far below humans in *scala naturae* (Aristotle, 1907). The topic of animal sentience has been central to the welfare debate. René Descartes (1596-1650) famously claimed that animals were soulless and non-sentient, and therefore like machines (Wilson, 2002). Jeremy Bentham (1748-1832), by many regarded as one of the first supporters of animal rights, just as famously asked "the question is not, Can they reason? nor, Can they talk? but, Can they suffer?" (Bentham, 1907).

In more modern time, Ruth Harrison is said to be the person who introduced the animal welfare debate to Europe. In 1964 she wrote the book «Animal Machines» where she described the intensive farming of poultry and other livestock. Partly based on the public reactions to the book, the UK Government commissioned an investigation into the welfare of intensively farmed animals, led by Professor Roger Brambell. The investigation resulted in a recommendation stating that all animals should have the freedom to "stand up, lie down, turn around, groom themselves and stretch their limbs". This short list became known as Brambell's Five Freedoms. The freedoms, a set of right for animals under human control, have later been developed by the Farm Animal Welfare Committee (FAWC) and are currently expressed as:

1. **Freedom from hunger or thirst** by ready access to fresh water and a diet to maintain full health and vigour

2. **Freedom from discomfort** by providing an appropriate environment including shelter and a comfortable resting area

3. **Freedom from pain, injury or disease** by prevention or rapid diagnosis and treatment

4. **Freedom to express (most) normal behaviour** by providing sufficient space, proper facilities and company of the animal's own kind

5. **Freedom from fear and distress** by ensuring conditions and treatment which avoid mental suffering

The five freedoms have been modified and incorporated into various national legislations as well as the EU legislation (Lundmark et al., 2014). They have also been adopted by influential groups worldwide, including the World Organisation for Animal Health (OIE) and form the basis of what constitutes good animal welfare (OIE - World Organisation for Animal Health, 2010).

The industrialisation of the primary production over the last 50 years, focusing almost exclusively on biological functioning and production, has resulted in serious threats to animal health and welfare (D'Silva, 2009). During recent years there has been a shift towards a greater focus on animal welfare, including their affective state and natural living.

Defining animal welfare

Despite its long history, there is no *one* definition for animal welfare. Rather, animal welfare is defined according to what emphasis one chooses (Fraser et al., 1997) (Figure 1). Traditionally, animal welfare has been associated with biological functioning. This view holds that an animal in good health with normal production and reproduction also has good animal welfare. A second approach to the animal welfare concept is that the subjective state of the animals determines its level of welfare. A third approach encompasses naturalness. Supporters of this direction stress that the animals should be able to lead natural lives and be given the opportunity to express their natural, innate behaviours (Fraser et al., 1997). The first approach is often advocated among people involved in livestock farming. The second approach is often held by humanitarians, while the third is often emphasised in organic production, by the public and by those critical of intensive farming (Fraser, 2003).
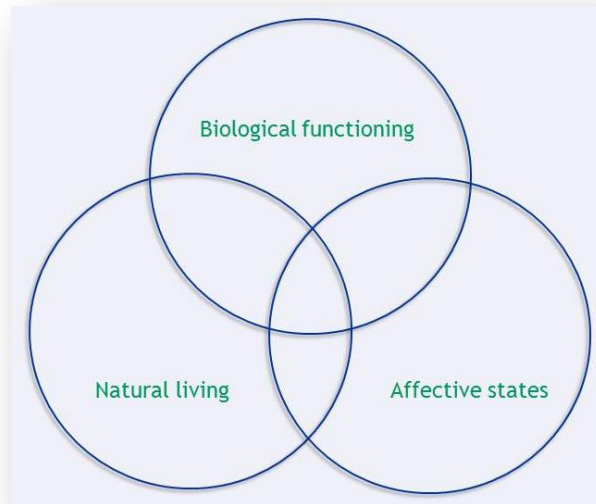
Figure 1. Three approaches to animal welfare. Figure by Lund (2002).

Although there is no consensus regarding the definition of animal welfare, a number of definitions exist:

Saunders Comprehensive Veterinary Dictionary defines animal welfare as "the avoidance of abuse and exploitation of animals by humans by maintaining appropriate standards of accommodation, feeding and general care, the prevention and treatment of disease and the assurance of freedom from harassment, and unnecessary discomfort and pain (Blood and Studdert, 1999).

Donald Broom is the author of another definition saying that "The welfare of an individual is its state as regards its attempts to cope with its environment" (Broom, 1986). Coping is generally thought to include health, physiology and behaviour, and how well the coping succeeds, determines its level of welfare (Broom, 1991). Broom is generally viewed as a supporter of the biological functioning approach, but has also incorporated the aspect of affective states (Broom, 1998).

Marian Dawkins, a professor in animal behaviour and welfare, has a simple approach to the concept. By asking the questions "Are the animals healthy?" and "Do the animals have what they want?" their level of welfare can be determined (Dawkins, 2004). Dawkins is commonly seen as promoting the affective state approach.

OIE states that "Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress. Good animal welfare requires

disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing" (OIE - World Organisation for Animal Health, 2010).

As can be seen in the definitions above, different emphasis gives different definitions. In practice there is great overlap among the three approaches and it is hard to discuss animal welfare without coming back to all three. All three approaches are also incorporated into the Norwegian animal welfare legislation, which is in place to safeguard animal welfare.

Animal welfare legislation in Norway

Norway has a tradition of strict animal welfare legislation and is generally viewed as having high levels of animal welfare. Norway, along with the rest of Scandinavia, early introduced welfare legislation. Already in 1842 the Norwegian criminal code (Straffeloven) contained a ban on animal abuse, but only the most severe cases were prosecuted. A comprehensive animal welfare act was introduced in 1935, which was replaced by a second welfare act in 1974. The Animal welfare act in its current version entered into force in 2010. This act is unique in that it holds that "Animals have an intrinsic value which is irrespective of the usable value they may have for man" (Landbruks- og matdepartementet, 2010).

The welfare act is made up of generic principles. More detailed requirements are given in species-specific directives. For instance, in the directive for cattle keeping it is stated that calves older than eight weeks cannot be kept in single boxes or pens if there are other calves at approximately the same age in the herd (Landbruks- og matdepartementet, 2004). The calves must be allowed to see and physically touch other animals. It is also stated that calves should be fed at least twice a day and be given sufficient amounts of colostrum as soon as possible after birth (Landbruks- og matdepartementet, 2004). Norway is not a member of the European Union (EU), but through the European Economic Area (EEA – EØS in Norwegian) Norway is bound to follow the EU regulations.

Animal welfare legislation forms the foundation for animal welfare. However, implementation is often slow due to political and economic considerations (Appleby, 2004). Also, as values differ, so will the level of animal welfare, although based on the same legislation (Lundmark et al., 2014). The decisions and actions a farmer take to comply with animal welfare legislation and at the same time maintain animal welfare according to personal standards, is called management.

Calf management

Defining management

The Saunders comprehensive veterinary dictionary defines management as the "Technique, practice or science of managing or controlling; the skilful use of resources and time; the specific treatment of a disease or disorder" (Blood and Studdert, 1999). In other words, management is how you treat your animals, how you divide your time and perceive and deal with adverse events. The list of management practices is long and, with respect to calf management, includes all aspects of the calf's life.

Management practices

Broom and Fraser (2007) list a number of basic needs that need to be maintained through management. They include feed and drink, rest and sleep, exercise, lack of fear, the ability to explore, minimal disease, ability to groom and avoidance of pain. Certain management practices are very important to animal welfare and need extra attention. One such practice is colostrum feeding. Calves should be given sufficient amounts of colostrum as soon as possible after birth to ensure a good immune status. Colostrum is high in immunoglobulins (Ig, predominantly IgG), which protects against infectious diseases. Diarrhoea (Svensson et al., 2006) and respiratory disease (Gorden and Plummer, 2010) are often caused by viruses and constitute the two greatest health threats for young calves. Figures from the Norwegian Cattle Health Recording System (NCHRS) show that these two conditions make up almost 80 % of all registered disease treatments in calves (Gulliksen, 2010). Besides pathogens, the presence of diarrhoea is influenced by a number of management factors, including hygiene, number and density of animals and housing of calves (Klein-Jöbstl et al., 2014).

Calves are social animals. To support their wellbeing, single calf pen walls must be created in such a way that the calves can see and physically touch each other when standing. Dairy calves housed in pairs and fed higher amounts of milk have also been found to have greater body weight gain and increased play behaviour (Jensen et al., 2015), an indicator of good animal welfare. According to the Norwegian regulations, the housing conditions must allow the calf to lie down and get up as well as perform grooming in a natural way (Landbruks- og matdepartementet, 2004). The regulations also state that the resting area

should be draught free and clean. The flooring should have sufficient bedding, providing a soft lying area.

Management challenges

Unfortunately, calves often get the short end of the stick when new barns are designed (Gulliksen, 2010), probably because it is easier to see the direct economic benefit of the adult cow. E.g. placing calf pens in a draughty area of the barn can affect the animals' disease resistance. Also, housing young calves with older animals can expose calves to pathogens and affect their health and welfare (Assié et al., 2009). Further, structural rationalization may influence calf welfare in a number of ways. Increasing herd size, increasing number of joint operations ("samdrift") and loose housing systems may all increase the risk of calf morbidity and mortality (Gulliksen et al., 2009a; Gulliksen et al., 2009b; Østerås, 2009).

A higher number of animals per herd will also influence the human-animal relationship (HAR). While few animals and close contact between animal and stockperson used to be the standard, with an increasing number of animals, the time to handle each individual animal decreases (Raussi, 2003). Also with loose housing units there is more space for the animals to evade people, potentially making them more difficult to handle (Raussi, 2003).

The complexity of management

Calf management is influenced by many factors ranging from the behaviour and habits of the individual farmer to the traditions, guidelines, rules and legislation communicated within the production system. These factors interact and influence each other, making calf management a complex matter. Recently deceased British philosopher Roy Bhaskar (1944 – 2014) describes this complexity between the individual and the structural level of the society in his transformational model (Bhaskar, 2014). Bhaskar holds that a society is a network of relations between individuals. The structure in society, he claims, is both a condition for, and results of human action. This is because individual actions always exist within a structure, at the same time as the structure depends on its reproduction through human activity. Therefore, society cannot exist without individuals and individuals cannot exist without society (Bhaskar, 2014). One non-calf related example of this duality is language. We don't speak to maintain the structure of language; we speak to interact with each other. However, as

language is the condition for speech, we maintain the system of language. At the same time, language is the results of individuals' acts of speaking. Through conscious speech, we also use the language in new ways. Consequently, the language develops (Bhaskar, 2014). This is also the case with calf management. The farmer operates within a structure that is both a condition for and the result of his/her actions, and calf management is therefore sustained and developed through this duality. This thesis therefore investigates how management may impact calf welfare at the societal and the individual levels described in Bhaskar's transformational model (Bhaskar, 2014).

One possible way of influencing animal welfare is through legislation, which provides the framework in which management is performed. The two main production systems in Norway are conventional and organic production. The systems each have specific regulations and values. Hence, the prerequisites for management are different.

Dairy production in Norway

Conventional dairy production

In January 2015 there were 222 000 milking cows divided among just under 9 000 dairy farms in Norway (Tine, 2015). In line with Norwegian politics, milk is produced on dairy farms all over the country. With an average of 25 milking cows per producer, Norwegian farms are generally small on an international scale. In Denmark, for instance, the average number of dairy cows per herd is 160 (The Dairy Site, 2013). In the US, the number is just short of 200 (USDA Milk Production Report, 2014). Although milk is produced nationwide, the Norwegian dairy industry has undergone a structural rationalization over the last 20 years. As has also been the trend internationally, the number of producers has decreased and the number of animals per unit has increased. Joint operations have also been politically encouraged through financial subsidies. Many producers have also invested highly in their establishments, with state-of-the-art automated equipment. For instance, with about 1500 milking robots, Norway has the highest share of automated milking systems (AMS) per producer in the world (Nodeland, 2013).

The major dairy cattle breed in Norway, constituting 94 % of the national herd, is the Norwegian Red (Tine, 2015). This is a dual purpose breed used both in milk and meat production. Each cow produces on average 7600 kilograms of milk per year. Traditionally, Norwegian cattle have been housed in tie stalls. As legislation now prohibits new tie stall

barns to be built, a transition is taking place in which an increasing number of cows are being housed in loose housing units. By 2024, all Norwegian cattle must be housed in loose housing systems, as a total ban on tie stalls will be introduced with the exception of organic farms with less than 35 cows (Landbruks- og matdepartementet, 2008).

Organic dairy production

Organic farming has formally existed in Norway since the 1970s (IFOAM, 2014). Since then, organic food production in Norway as in the rest of Europe has been steadily increasing. Still, the national organic production is small. The proportion of organically farmed cattle in Norway in 2013 was only 3.7% (Statens landbruksforvaltning, 2013). The number of organic producers in Norway has also declined during the last year, as is the overall trend for dairy producers. Organic groceries constituted 1.2% of all groceries sold in 2013, with dairy products comprising 21% of the 1.2% (Statens landbruksforvaltning, 2013). It has long been a political goal in Norway to reach 15% organic production by 2020 (Landbruks- og matdepartementet, 2009) and make organic farming the leading way of farming. Reaching these goals is proving difficult.

Organic dairy production and animal welfare

The basic philosophy of organic farming is to develop robust, sustainable and environmentally friendly production systems, while at the same time focusing on animal health and welfare (Rigby and Cáceres, 2001; Shi-ming and Sauerborn, 2006; IFOAM, 2009). Organic production has a stronger emphasis on natural living, including the possibility to perform natural behaviours and providing a natural environment for the animals than conventional production (Lund et al., 2002; Lund, 2006). This is reflected in the specific regulations for organic farming. For instance, the animals are allowed more space, they are encouraged to spend more time outside and the calf must be allowed to stay with the dam for at least three days after parturition (Mattilsynet, 2015). The regulations are generally stricter than the rules for conventional production and involve all aspects of the production including housing, feeding, cow-calf separation, outdoor areas, and medicine use.

Although the organic production systems has a legislation and a philosophy that form a solid foundation for good animal welfare, organic farmers' individual motivation (Lund et al., 2004b) and management practices are highly variable. Veterinarians have been accused of

having little knowledge about the organic standards and welfare philosophy (Vaarst, 1998) and of being unrightfully sceptical towards organic production (Sundrum et al., 2006; Vaarst et al., 2006; Vaarst et al., 2008). Whether the rules and regulations of organic farming provide better animal welfare compared to conventional dairy farming, needs to be investigated.

An old saying dictates "You can tell what kind of a stockman a person is by looking at his cattle". This implies that even the best production system can fail if not managed by a good stockperson. The human care factor is generally viewed as one of the most significant factors in determining the level of welfare in domestic animals (Hemsworth and Coleman, 1998; Rushen et al., 1999b). How the farmer makes "use of resources and time" (Blood and Studdert, 1999) and interacts with his/her animals, forms the basis for the human-animal relationship.

Human-animal relationship (HAR)

The concept of HAR

HAR can be defined as "the degree of relatedness or distance between the animal and the human, i.e., the mutual perception, which develops and expresses itself in their mutual behaviour" (Estep and Hetts, 1992). It is stated in the Norwegian regulations that cattle should have proper level of tameness and that calves must be habituated to people from an early age (Landbruks- og matdepartementet, 2004). The major factor influencing the HAR is the nature of the daily interactions between the stockperson and the animal (Hemsworth et al., 1981a; Hemsworth et al., 1981b), as stockperson behaviour determines the animals' reaction towards humans and, hence, the quality of the HAR (Waiblinger et al., 2006; Zulkifli, 2013). A good HAR is therefore fundamental to good animal welfare.

It has been known for some time that a good HAR also has a direct effect on production. As early as in the 1970s, it was reported that «herds where the cows readily approached the herdsman had a significantly higher yield per cow than herds where the cows lacked this confidence» (Seabrook, 1972). Still, the study of the effects of stockmanship has only during recent years become a prioritized research area.

The role of the stockperson

Stockmanship can be defined as "knowing the behaviour pattern of animals and groups of animals within one's charge and having the ability to recognise small changes in the

behaviour of any one animal or of all the animals collectively" (Seabrook, 1986). Stockpeople are professional managers of animals, fundamental in determining animal performance and welfare (Hemsworth and Coleman, 1998). Stockperson qualities are therefore not to be underestimated. In a recent paper by Coleman and Hemsworth (2014) three main factors are listed as contributors to a stockperson's work performance: Capacity, willingness and opportunity. Capacity involves skills, health, ability and knowledge. Willingness includes motivation, job satisfaction and attitude, while opportunity includes working conditions, actions of co-workers as well as organisational policies and rules (Coleman and Hemsworth, 2014).

The direct effect of stockperson personality on animal production, health and welfare is debatable (Seabrook, 1972; Panamá Arias and Špinka, 2005; Hanna et al., 2009), but it is clear that different traits can influence worker performance. For instance, a stockperson scoring high on conscientiousness may be good at accomplishing tasks. A person scoring high on extraversion may prove proficient in training (Hemsworth and Coleman, 1998). Among other influential traits are how close the stockperson feels towards the animals (Marinelli et al., 2007), how the persons view the cognitive abilities of the animals (Davis and Cheeke, 1998), gender (women often supposed to be better than men (Raussi, 2003)), and culture. Seabrook (1986) also states that a good stockperson talks to and touches the animals, enjoys being with them and spends more of the available time with the animals. A good stockperson also touches and communicates more with the animals when they are under stress (Seabrook, 1986). Among all the traits influencing the HAR, attitudes have been found to be the most consistent predictor of stockperson behaviour (Breuer et al., 2000; Waiblinger et al., 2002; Hanna et al., 2009). Several authors also list the farm manager's attitudes as the most important factor determining the quality of animal handling (Grandin, 2000).

The relationship between the stockperson and the animals can be as depicted below (Figure 2). The attitudes of the stockperson influence his/her behaviour. This will again decide the level of fear the animals experience towards humans, which again influences their level of productivity and welfare. There is also a feedback loop running back to attitudes. This means that the stockperson's own behaviour, as well the outcome of the behaviour, will feed back on the stockperson's attitudes.
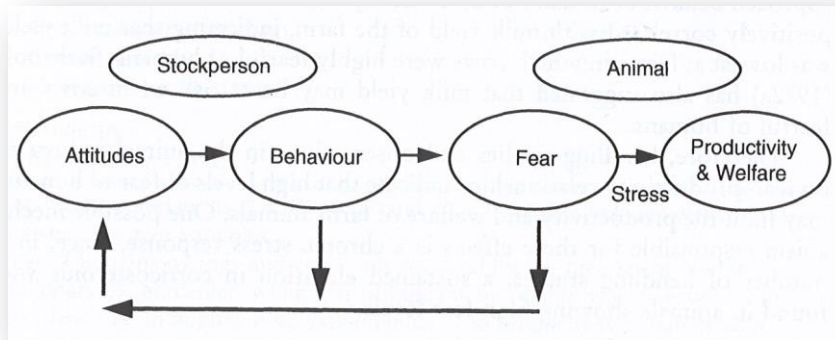
Figure 2. A model showing the relationship between humans and animals in animal production (Hemsworth et al., 1993).

Measuring HAR

There are a number of ways to measure the quality of the HAR. If you want to investigate the stockperson, attitudes or behaviour can be recorded. Attitudes cannot be measured directly, but can e.g. be operationalized through the use of a questionnaire (Hemsworth and Coleman, 1998). The farmer's behaviour can also be observed directly during routine tasks like milking or moving animals (Waiblinger et al., 2006).

If you want to study the HAR from the animal point of view, de Passillé and Rushen (2005) suggest three types of measures: (1) Distance measures to see how much the animal approaches or avoids people. (2) Handling measures to assess the animal's response to being handled. (3) Rating scales involving some sort of subjective rating of the animal, like personality or temperament (de Passillé and Rushen, 2005). In addition, physiological measures like the animal's heart rate, blood corticosteroid levels, morbidity and productivity may be used (Hemsworth and Coleman, 1998).

A novel way of investigating the HAR is through Qualitative Behaviour Assessment (QBA). QBA is an integrated assessment of the whole animal where the animal's body language is evaluated as an indication of the animal welfare state (Wemelsfelder and Lawrence, 2001). Originally from the field of human psychology on non-verbal communication, QBA has been introduced into the field of animal welfare research by Francoise Wemelsfelder (Wemelsfelder, 1997; Wemelsfelder et al., 2000).

If a method such as the QBA is to be used for animal welfare assessment in an experimental or on-farm setting, validity, reliability and feasibility needs to be established. Over the last 15 years, QBA has therefore undergone testing in an attempt to determine its

validity in terms of its correlation with quantitative and physiological indicators of animal welfare, its inter- and intra-observer reliability (Table 1), as well as its feasibility. Wemelsfelder herself has authored or co-authored most of these publications.

As Rutherford et al. (2012) explain, the validity of a measurement tool can never be fully established. Rather, new studies are conducted that influence the degree of confidence we can place on that method (Rutherford et al., 2012). The validation process of QBA is on-going, and a number of studies have shown that QBA has meaningful relationships with behavioural and physiological welfare measures. Comparing QBA scores with quantitatively recorded behaviours, e.g. Rousing and Wemelsfelder (2006), Napolitano et al. (2007), Minero et al. (2009), Rutherford et al. (2012), and Napolitano et al. (2012) found significant relationships in dairy cattle, horses and ponies, foals, pigs, and dairy buffaloes, respectively. Although the number of studies comparing QBA scores to physiological indicators is relatively scarce, Rutherford et al. (2012) found QBA to be significantly correlated with core body temperature, heart rate, plasma glucose, and neutrophil:lymphocyte ratio in pigs. Stockman et al. (2012) also found cattle with a greater plasma lactate concentration to be classified as more nervous/anxious, and an animal with an ultimate muscle pH >5.7 to be more annoyed/frightened, using QBA. A study on habituation to transport in sheep also found QBA scores to be significantly correlated to heart rate, heart rate variability, core body temperature and a stress leukogram (Wickham et al., 2012). On the other hand, Brscic et al., (2009) found no relationship between QBA and a clinical/health evaluation protocol in veal calves, except for cross-sucking.

Several studies have found that QBA can detect treatment effects. Examples include housing in an unenriched vs. enriched environment in pigs (Wemelsfelder et al., 2000), extensive vs. intensive rearing conditions in pigs (Temple et al., 2011), a familiar indoor pen vs. a novel outdoor pen in dairy buffaloes (Napolitano et al., 2012), habituation to handling in foals (Minero et al., 2009), the presence of unfamiliar people in veal calves (Brscic, et al., 2009), habituation to transport in beef cattle (Stockman et al., 2011) and dairy cattle (Wickham et al., 2012), the place in line for slaughter in beef cattle (Stockman et al., 2012) and treatment with a sedative drug in pigs (Rutherford et al., 2012).

Before a new method can be applied, establishing high levels of inter- and intra-observer reliability is crucial. Table 1 shows the inter- and intra-observer reliability of QBA in publications from 2000 to 2015 in different animal species.

Table 1. Publications assessing the inter- and intra-observer reliability of Qualitative Behaviour Assessment in different animal species. Inter-observer reliability for free-choice profiling is shown by the level of consensus among observers (in per cent) after performing a General Procrustes Analysis. The significance test is done by testing whether the consensus profile is significantly different from a random profile. Inter-observer reliability for fixed terms is shown by Kendall's W correlation, while intra-observer reliability is shown by Pearson's R or Spearman rank correlations.

Publication	Species	Inter-obs. reliability	Intra-obs. reliability
Wemelsfelder et al., 2000	Pigs	81.0** (Part 1) 84.8** (Part 2)	
Wemelsfelder et al., 2001	Pigs	79.9-81.1** (live) 85.3** (video) 72.6-74.5** (merged)	0.88-0.99**
Rousing and Wemelsfelder 2006	Dairy cattle	66.4-71.4** (video) 64.4** (merged)	0.96** (Dim1) 0.95** (Dim2)
Napolitano et al., 2007	Dairy cattle	Value not given**	
Napolitano et al., 2008	Horses Ponies	78.8**	
Minero et al., 2009	Foals	58.4**	
Wemelsfelder et al., 2009a	Laying hens	0.83** (PC1) 0.73** (PC2)	
Wemelsfelder and Millard 2009	Pigs	0.82** (PC1) 0.56**(PC2)	
Wemelsfelder et al., 2009b	Beef cattle	0.73** (PC1) 0.84**(PC2)	
	Dairy cattle	0.38 (p<0.06) (PC1) 0.46* (PC2)	
	Veal calves	0.64** (PC1) 0.40* (PC2)	
Wemelsfelder et al., 2009c	Pigs	70.2**	
Brscic et al., 2009	Veal calves	Rearing environment not important for reliability	
Walker et al., 2010	Dogs	78.8**	
Müllner 2011	Dairy cattle	58.0** (Free-choice) 56.9** (Fixed)	
Stockman et al., 2011	Beef cattle	47.0**	
Rutherford et al., 2012	Pigs	49.7**	
Bokkers et al., 2012	Dairy cattle	Slight to moderate (QBA scores)	0.71-0.88*
Napolitano et al., 2012	Dairy buffaloes	77.1-77.9**	

Table 1 continues on the next page

Wemelsfelder et al., 2012	Pigs	69.0-76.0**	0.86-0.99**
Stockman et al., 2012	Beef cattle	43.7**	
Wickham et al., 2012	Sheep	53.0**	
Andreasen et al., 2013	Dairy cattle	0.72*** (PC1) 0.56***(PC2)	
Phythian et al., 2013	Sheep	0.78-0.90** (PC1) 0.84-0.91** (PC2)	
Fleming et al., 2013	Horses	47.9**	
Gutmann et al., 2015	Dairy cattle	0.37* (morning) 0.28-0.29* (late morning) 0.46-0.50* (afternoon)	

▭ = Free-choice profiling ▭ = Pre-fixed list of descriptors ▭ = Free-choice and pre-fixed list

*p<0.05, ** p<0.001, *** p<0.0001

As evident from Table 1, most, but not all papers report high inter- and intra-observer reliability for QBA.

QBA is a non-invasive method which does not require any equipment and is relatively quick to apply (Stockman et al., 2012; Andreasen et al., 2013). It is also a technique that can be applied by a wide range of people and professions, including animal welfare inspectors, veterinarians and stockpeople themselves. Despite different backgrounds and experiences, people have been found to reach consensus in their assessments using QBA (Wemelsfelder et al., 2012).

Although QBA may not give sufficient information about the welfare state of animals to replace other measurements, it may form part of a welfare protocol (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001) or function as a first level of inspection to identify producers in need of a second, more thorough inspection (Brscic et al., 2009). Several authors (Wemelsfelder and Rousing, 2006; Brscic et al., 2009; Temple et al., 2011; Rutherford et al., 2012; Stockman et al., 2012) also recognize the potential of QBA to provide additional information about the welfare state of the animals, complimentary to the information gained by other measures. One such example is physical activity. In the study by Rutherford et al. (2012) physical activity is included as a quantitative measurement. However, as Rutherford et al. debate, if no assessment of emotionality is included, it is impossible to know if the observed walking signifies confidence and curiosity, or agitation and escape behaviour.

Due to the generally high levels of validity, reliability and feasibility, QBA has the potential to provide information about animal welfare. Applying QBA to investigate the HAR, however, has never been done before.

In dairy production today, a number of management procedures are routinely carried out. Two such procedures that greatly influence calf welfare are cow-calf separation and artificial milk feeding. The procedures will be further investigated.

Cow-calf separation

Natural behaviour and current practice

Cattle are gregarious animals with strong maternal behaviour (Jensen, 2002). Under natural conditions, cow and calf establish strong bonds and remain together until the calf is gradually weaned at approximately 6-8 months (Phillips, 1993). In conventional dairy production, the animals are normally not allowed to bond as cow and calf are usually separated immediately after birth. Even calves raised in organic dairy production, a production system promoting natural behaviour (IFOAM, 2012), are only required to stay with their dam for three days in Norway (Debio, 2005), or one in Sweden (KRAV, 2015). The rules for organic production in the European Union simply state that calves "shall be fed on maternal milk in preference to natural milk, for a minimum period of three months" (European Commission, 2008). There are no specifications regarding suckling or staying with the mother.

If allowed, the cow will usually isolate herself from the rest of the herd shortly before calving (Jensen, 2002). This is probably a way of enhancing recognition and attachment between the mother and calf, further aided by licking, grooming, smelling, tasting and looking at the calf (Broom and Fraser, 2007). Calves are natural hiders, meaning they don't follow their mothers around the first days after birth. Rather, they stay hidden until the dam returns and they may suckle. Periods of separation from the cow are therefore natural for the young calf (Weary et al., 2008). Nevertheless, prolonged separation may lead to a number of physiological and behavioural responses.

Response to separation

When animals that are strongly bonded and motivated to regain contact are prematurely separated, they perform reinstatement behaviour including vocalization and

locomotion (Panksepp, 1998). Frequencies and durations of these behaviours can hence be used to evaluate the level of stress associated with separation. Both cows and calves have been found to vocalize a lot during the first days following separation (Lidfors, 1996; Marchant-Forde et al., 2002; Haley and Stookey, 2005). This is thought to be a reaction to the separation between the two (Loberg et al., 2008). Cow and calf vocalize when they are together as a way of improving the maternal bonding (Marchant-Forde et al., 2002). However, the high pitched vocalization performed with open mouth, has been described by many as a response to separation (Lidfors, 1996; Weary and Chua, 2000), either by very hungry animals or by cows that have lost their calf (Kiley, 1972). It is therefore probable that this vocalization indicates frustration. The calves' cardiac response has also been found to be affected by the cow's vocalization at separation (Marchant-Forde et al., 2002), indicating that the welfare of the calf can be affected by the dam's reaction.

Other separation reactions indicative of stress can be cow and calf fence-line pacing in the days following separation (Haley, 2006; Enríquez et al., 2010). This is likely to be a behaviour aimed at reuniting cow and calf. The same is true for placing the head outside the pen (Flower and Weary, 2001; Loberg et al., 2007; Stěhulová et al., 2008). Further, cows have been found to ruminate less often immediately after separation (Lidfors, 1996). It has also been shown that calves only allowed auditory contact with the dam after separation showed more alert behaviour, including behaviours like gazing and directing ears, head and neck in the direction of the cow (Enríquez et al., 2010).

As separation is a major welfare challenge both for cow and calf leading to a variety of behavioural and physiological reactions, a number of studies have been dedicated to developing alternative management methods to reduce post-separation stress.

Alternative separation methods

One such alternative method is fence-line (FL) separation. Originally developed for beef cattle, which are generally weaned by separation, FL separation implies that the cow and calf may have limited physical contact over/through a fence in the days following separation (Nicol, 1977). This way, the calf may continue to receive milk artificially after being separated from the dam, but is prevented from suckling. Another alternative is to separate by two-step weaning. Using this method, the calf is allowed to stay with its dam, but is prevented from suckling, e.g. by using a nose flap or an udder net. A potential advantage of both these methods is that weaning and physical separation occur at different points in time.

In addition to the alternative separation methods, there are certain management measures one may take to help ease the transition. Feeding calves more milk at higher frequencies during the days after separation has been found to reduce vocalization in calves (Thomas et al., 2001). It can also be advantageous to separate more than one calf at a time, as calves with full social contact establish strong bonds with their companions (Duve and Jensen, 2011) which again has been found to reduce stress (Duve et al., 2012).

As a result of early separation of cow and calf, dairy producers must artificially milk feed their young calves. The management of milk feeding, however, is controversial, especially in relation to the amount of milk farmers feed their calves.

Milk feeding

The importance of colostrum

As opposed to animals like dogs, cats and humans, there is no transfer of passive immunity from the cow to the calf during pregnancy. The calf is therefore dependent on a sufficient volume of good quality colostrum, the first milk after calving which is high in immunoglobulins (IgG, IgM and IgA). The colostrum is also important as the first source of nutrition after birth. It is essential that the calf receives colostrum as soon as possible after birth, preferably within two hours after parturition (Godden, 2008). The ability of the gut to absorb the antibodies in the colostrum drop rapidly over the first few hours of life and after about 24 hours the absorption is practically non-existent (Bush and Staley, 1980). Calves are defined as having failure of passive transfer (FPT) if the calf serum IgG concentration is below 10 mg per mL when the calf is between 24 and 48 hours old (Godden, 2008).

Providing the young calf with sufficient amounts of milk is important when separating cow and calf. Also later, the nutritional status of the calf is based on milk feeding and has a great effect on immune status and hence health and welfare.

The calf digestive system

The calf has an anatomical feature called the oesophageal (reticular) groove (*sulcus retículi).* When the oesophageal groove reflex is activated, the muscular walls of the groove contract and form a pipe. This action shunts the ingested milk past the reticulum-rumen-omasum into the abomasum (Sjaastad et al., 2010).

It is commonly believed that young calves should not be fed more than about two litres of milk per meal. Milk meals beyond this volume, can allegedly cause milk to enter the rumen. This can either occur as a result of insufficient closure of the oesophageal groove (Borne et al., 2004) or due to backflow from exceeding the capacity of the abomasum (Akssenowa, 1931; Borne et al., 2004; Smith, 2014). For the youngest calves, this may not pose a problem. The forestomachs are so little developed that any milk in the rumen simply empties into the abomasum within a few hours (Lateur-Rowet and Breukink, 1983). For older calves with a more developed rumen, milk in the forestomachs may be problematic. The lactose is converted to lactic or other organic acids, or the milk protein may rot, which again can affect the rumen micro flora and cause indigestion, diarrhoea and reduced growth (Sjaastad et al., 2010). The volumes, at which the abomasal capacity is exceeded causing milk to enter the rumen, are not found to be scientifically addressed.

Knowledge gaps

As has become evident in the various topics presented, certain knowledge gaps exist in the field of calf management and its impact on welfare. Some of these gaps are addressed in this thesis.

Firstly, a more thorough investigation of calf health and welfare in organic dairy production in Norway is needed. Although a small production system in Norway, organic dairy production has a focus and a philosophy promoting natural behaviour. Is this alternative management form in itself an indication of higher welfare? What are perceived to be the main advantages and disadvantages of organic farming in relation to calf health and welfare?

Secondly, the topic of stockperson behaviour deserves further attention. Does the behaviour of the farmer directly influence calf behaviour and welfare?

Thirdly, there are gaps in the knowledge regarding alternative management practices for separating cow and calf after a suckling period. Are there ways of making separation of cow and calf less stressful to the animals?

Fourthly, the general acceptance that calves cannot be fed more than 2-3 litres of milk per meal is a major reason for restrictive milk feeding in dairy calves. The traditional milk meal size limit has an unclear origin and it is therefore unknown whether this management practice is valid. Can we increase the milk meal size in young calves beyond 2-3 litres, and if so, how large milk meals can they receive before milk ends up in the rumen and causes health and welfare problems?

Aims of the thesis

The overall aim of this thesis was to contribute knowledge related to the impact of management on dairy calf welfare. This was achieved through four studies with the specific objectives as follows:

1. Describe practicing veterinarians' and agricultural advisors' impressions of calf health and welfare in organic dairy farming.
2. Measure what effect stockperson behaviour has on calf behaviour.
3. Compare two management methods for separation – with or without physical contact between the calf and dam – to see if the opportunity for physical contact reduces the separation distress in cows and calves.
4. Define how much milk a dairy calf can drink from a teat bottle in one meal before the milk enters the rumen or causes abdominal pain or discomfort.

Materials and Methods

Paper I – Questionnaire survey

Data collection

Paper I is based on an Internet-based questionnaire. The questionnaire was created and conducted in 2008 using QuestBack™. First, the survey was sent out to all members of the Norwegian Association of Large Animal Veterinary Practitioners registered with an email address. This organization constitutes approximately 90% of all large animal practitioners in Norway and the number of recipients amounted to 400. Second, the survey was sent out to an equally large group of 400 agricultural advisors. The advisors were all employed by Tine, Norway's dominant producer and distributor of dairy products, owned by the producers themselves. A total of 207 veterinarians (52%) and 215 agricultural advisors (54%) responded to the questionnaire. Of these, 114 veterinarians and 112 advisors had experience from organic farms during the last five years and were included in the analyses.

The questions in the survey were aimed at covering central aspects of calf health and welfare, with emphasis on organic dairy production. Input from experts during two workshops on calf health and welfare aided the development of the questionnaire. A draft questionnaire was also tested on a group consisting of three veterinarians and one agricultural advisor and modified according to their feedback to avoid any ambiguities.

The final version of the questionnaire consisted of 50 questions and was divided into five parts:

1. Own knowledge

Respondents were asked to rate their own level of knowledge of the organic regulations regarding dairy cattle husbandry and medicine use.
Scale: From one ("very poor") to six ("very well").

2. Calves in organic vs. conventional production

Respondents were asked to rate their impression of physical health, wellbeing, confidence in people, feed quality, feeding routines, hygiene and space allowance for calves on organic dairy farms compared to those in conventional dairy production.
Scale: From one ("much worse") through three ("equal to") to five ("much better").

3. General impression of calf welfare

Participants were asked to give their general impression of calf cleanliness, skin and coat condition, digestive and respiratory problems, inflammation of the navel and joints, deficiency diseases, body condition and growth, mortality, quality of treatment of ill and injured calves and maintenance of calf health card recordings on organic dairy farms.

Scale: From one ("very poor") to six ("very good").

4. Compliance

Participants were asked about the calves' opportunity to express natural behaviour, whether or not calves were separated from their mothers within 24 hours *postpartum* and whether calves had access to outdoor areas outside the grazing season

Scale: "Applies to all calves", "applies to most calves", "applies to some calves", and "applies to no calves".

5. Welfare advantages and challenges

Respondents were asked to choose what they considered the most important welfare advantage and the most important welfare challenge for calves in organic dairy farming.

Advantage alternatives: Calf kept together with cow, good stockmanship, a more natural life, social contact with conspecifics, space allowance, use of straw/soft bedding, good feeding routines, low disease rate and good indoor climate.

Challenge alternatives: Poor feed, inadequate treatment of disease, poor hygiene, alternative therapists without a veterinary degree, lack of sufficient follow-up, competition among calves, dirty calves and high disease rate.

For certain questions we wanted to test for potential differences within the sample. Participants were hence grouped according to profession (veterinarian or advisor), amount of experience with organic farms (1-3 vs. >3 farms) and years since graduation (<3 vs. >20 years).

Statistical analysis

Wilcoxon rank-sum tests (Gibbons, 1993) were performed to test for potential differences between the groups with respect to how well they knew the regulations concerning organic dairy cattle husbandry and medicine use.

Potential differences between organic and conventional farming systems were tested for by Wilcoxon signed-rank test (reference category 3 – "equal to") (Gibbons, 1993). The Wilcoxon rank-sum test was also used to test for effects of profession, exposure to organic farms and years since graduation on how respondents ranked the factors related to calf health, welfare, morbidity and mortality.

To test whether the veterinarians and advisors had rated the welfare advantages or challenges differently, a Pearson's chi-squared test (Gibbons, 1993) was applied to all statements with more than ten responses.

All comparisons were performed two tailed and differences considered significant for p-values below the level of 5%.

Paper II – Qualitative Behaviour Assessment (QBA)

Data collection

To collect data for Paper II, 110 Norwegian dairy farms were visited between January 2006 and March 2008. The farms included in the study were randomly chosen from a list of dairy producers in the Southern part of Norway. All farms were members of the NCHRS. During each farm visit, two Qualitative Behaviour Assessments (QBA) were performed: One on calves and young stock (QBA1) and one on stockpeople (QBA2). All behaviour registrations were done by the same observer, an experienced livestock inspector and agricultural advisor. The observer and one of the co-authors discussed and agreed upon the meaning of the QBA terms under the guidance of Dr. Francoise Wemelsfelder. The training consisted of farm visits where both the observer and the co-author scored the same animals independently. Comparisons of QBA scores were made, and if discrepancies were found, these were discussed. Comparisons and discussions were also performed during the data collection period to recalibrate and avoid drifting. The results were coinciding, although not quantified.

QBA1 was performed on calves and young stock up to 10 months of age. Most calves included in the study were Norwegian Red, but there were also some Norwegian Red cross

breeds, Jerseys, Simmental, Norwegian Red Polled Cattle, Blacksided Trønder and Nordland Cattle. The number of calves and young stock on the farms ranged from 10-120 and their age at the time of visit varied from 3-298 days. As multiple farm visits were conducted on the same day, standardising the time of the observations periods was not possible. However, care was taken to avoid feeding times. The QBA was carried out according to standard test procedure. The observer studied the animals for 10-20 minutes depending on the number of animals on the farm. If the farm only had a few calves and young stock, the observation period would be only 10 minutes. If the farm had a high number of animals requiring multiple observation points, the observation time was 20 minutes. Generally, all calves and young stock were observed. If this was not possible due to a high number of animals or other practical issues like housing at different locations, care was taken to ensure that all age groups were represented. After observing the animals, the observer went into another room and specified his level of agreement to each descriptor by indicating a position on a visual analogue scale (VAS). The VAS is different from a Likert scale in that the former is a continuous line, while the latter usually has discrete numbers, e.g. 1 - 6. The VAS is often used to measure subjective characteristics like attitudes and has been found superior to discrete scales (Reips and Funke, 2008). The 31 descriptors included in the calf QBA were: Nervous, frustrated, fearful, enjoying, distressed, uncomfortable, friendly, content, sociable, uneasy, calm, confident, agitated, unwell, happy, scared, positively occupied, relaxed, boisterous, inquisitive, playful, tense, aggressive, bored, depressed, active, lively, irritable, vigilant, apathetic, and indifferent.

QBA2 was performed on the person who was in daily contact with the calves and young stock on each farm. For the test procedure, the stockperson was asked to do a chest measurement on five calves successively. Subsequently, the data is based on 5 x 110 = 550 interactions. The five test animals were randomly chosen from a list of ear tag numbers. After the chest measurements had been performed, the observer scored, from memory, the body language of the stockperson interacting with the animals according to a list of 17 descriptors. During the handling, the stockperson was blind to the real purpose of the study, see Ethical considerations for details. The descriptors included in the stockperson QBA were: Quick, dominating, aggressive, fearful, patient, careful, calm, determined, focused , insecure, careless, talks to the animals, cuddles the animals, inventive, nervous, boisterous and including.

Both QBAs were performed during one visit. To avoid influencing the behaviour of the test animals, QBA1 was always performed before QBA2, as five of the animals observed in QBA1 were also used in QBA2.

Structural equation modelling (SEM)

SEM is a multivariate analysis technique used to test a conceptual or theoretical model (Byrne, 2010). The model is specified by the researcher based on an expected relationship between the variables. This expected relationship must be based on a priori knowledge and the theoretical basis for building the model should be given. The model can either be a *structural* model or a *measurement* model. The former shows potential causal dependencies between endogenous and exogenous variables. The latter shows the associations between latent variables and their indicators.

To test the model, a number of fit indices exist. The most common are:

- **Normed Chi square** (χ^2/df). A value < 2 indicates good fit (Schumacker and Lomax, 2004).
- **Comparative Fit Index** (CFI). A value < 0.95 indicate good fit (Byrne, 2010).
- **Root Mean Square Error of Approximation** (RMSEA). A value of zero indicates the best fit, values > 0.1 indicate poor fit (Byrne, 2010).
- **Standardized Root Mean Residual** (SRMR). Values < 0.08 indicate good fit (Hu and Bentler, 1999).

Statistical analysis

To analyse the QBA descriptors, the distance from the left-hand side of the VAS to the line drawn by the observer was measured, giving the score for each descriptor. The stockperson QBA was further analysed using Principal Component Analysis (PCA) with Varimax rotation (Quinn and Keough, 2002) on 10 descriptors to ensure maximum factor loading. The calf/young stock QBA was analysed according to the standard way of analysing animal QBAs (e.g. Andreasen et al., 2013; Phythian et al., 2013). Hence, all 31 descriptors were included in the PCA analysis and no rotation was performed.

The factor scores of each individual handling style and the factor scores of the two dimensions of calf/young stock behaviour, were used as variables in a SEM (Byrne, 2010) to

estimate the predicted relationship between stockperson handling style and the positive/negative mood of the calves and their level of arousal (Figure 3).
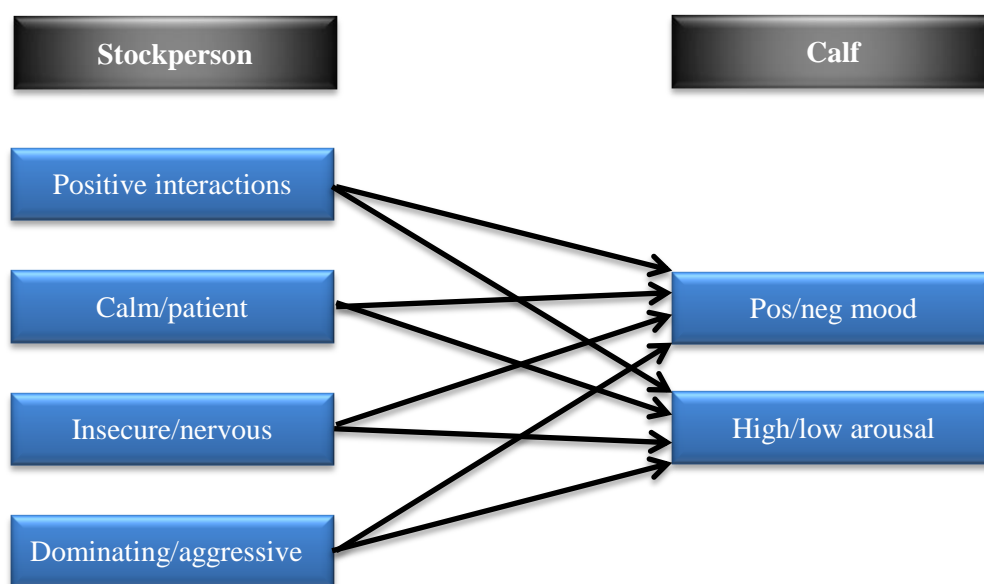
Figure 3. Structural equation modelling is used to investigate the relationship between stockperson handling style and calf mood and level of arousal. Figure by Kristian Ellingsen-Dalskau.

Paper III – Quantitative behaviour assessment

Experimental design

The data for Paper III was collected from one commercial Norwegian organic dairy farm between January 2010 and February 2011. All cows and calves in the study were of the Norwegian Red breed. Median cow parity in the trial was 2.5. The cows were fed silage and hay from a round bale feeder semi ad libitum and the animals had free access to water. At milking the cows were fed individual portions of concentrates. Only cows with an uncomplicated parturition were included in the study. Calves that were not observed to suckle within 6 hours after birth were assisted to do so according to standard farm practice. During the suckling period, calves were kept with free access to the cow pen and suckled the cows freely.

Eight batches of two cow-calf pairs were separated after 6-8 weeks of free suckling (mean ± SD: 54 ± 11 days) as part of the trial. Each batch consisting of two calves and their dams were randomly allocated to one of two separation methods; Fence-line (FL) or solid wall (SW).

FL-separation (8 cow-calf pairs) entailed that dam and calf were separated, but still allowed auditory, visual and tactile contact over and through a wooden plank fence erected between the indoor resting area and the calf pen (Figure 4). Suckling, however, was not possible.

SW-separation (8 cow-calf pairs) prevented the calf and the dam from seeing or physically interacting with each other as a two meter high, solid barrier was put up between the calf creep and indoor resting area (Figure 4). The cows and calves were, however, able to have auditory and possibly olfactory contact.

After separation, the calves were fed fresh, warm, whole milk from teat bottles three times per day (at 06.00, 14.00 and 16.30). The calves were hand fed and offered two litres of milk per meal. All calves accepted hand feeding within 24 hours. Cows were milked at 06.00 and 16.00 in an adjacent milking parlour.
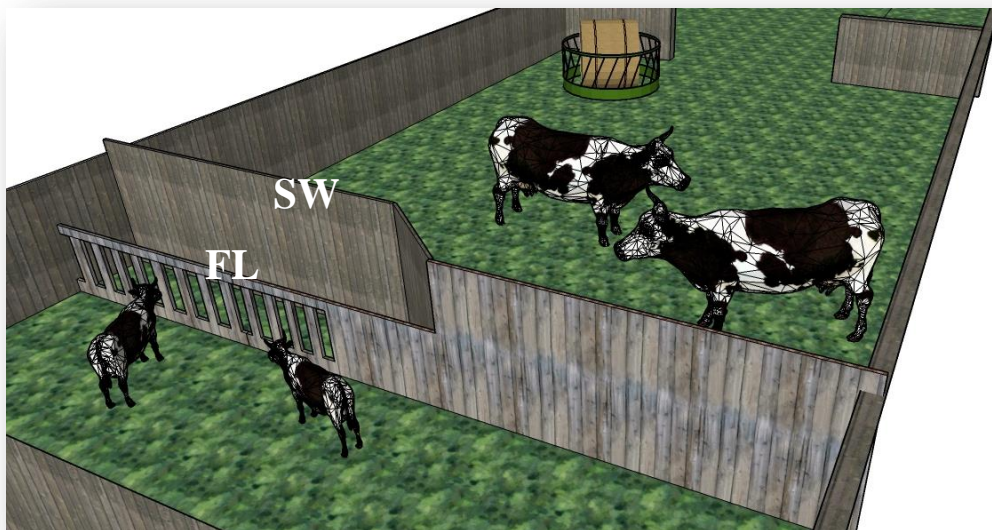
Figure 4. Diagram showing the setup of the separation treatments. In the solid wall (SW) treatment the cow-calf pairs were separated with a solid wall (SW) as depicted. In the fence-line (FL) treatment, the solid wall was removed and the cow-calf pairs were separated only by the low wooden plank fence. Figure by Kristian Ellingsen-Dalskau.

Behavioural observations

Direct observation was performed on the day of separation (day 0) and for four consecutive days after that (days 1 – 4). Each observation day comprised two 2-hour bouts, the first from 14.30-16.30 and the second from 17.00 -19.00. Thus, the calves were milk fed just prior to the observation periods. This was done as an attempt to keep the calves satiated at the time of observation. As concealment the observer was not possible, the animals were allowed 15 minutes to habituate to the presence of the observer prior to the start of every observation day. Blinding the observer to the treatment assignment was also impossible.

Behaviours believed to be an expression of post-separation stress were included in the ethogram (Table 2) and were either scored continuously or by using instantaneous sampling every 5 minutes. A line was painted on the wall on either side of the separation barrier indicating 2 metres (cow) or 1.5 metres (calf) for easy judgement of distance (Table 2).

Table 2. Ethogram showing behaviours recorded during direct, live observations of each focal cow or calf separated by fence-line (FL, n = 8 cow-calf pairs) or solid wall (SW, n = 8 cow-calf pairs). Behaviours are either scored continuously or by using instantaneous sampling every 5 minutes. The table has been reproduced with the kind permission of Journal of Applied Animal Behaviour Science, Elsevier.

Behaviour	Definition	Recorded for	Method of observation
High pitched vocalization[1]	Every single open mouthed "muh" vocalization with inhalation between two occurrences	Cow and calf	Continuous
Low pitched vocalization[1]	Every single closed mouthed "mm" type vocalization with inhalation between two occurrences	Cow and calf	Continuous
Head out of pen	Cow or calf puts its tip of the nose/head through openings- or over the fence or stands < 5 cm with any body part close to fence	Cow and calf	Continuous
Alert behaviour	Standing or lying down in any resting position with high-head posture, pointed ears with focus towards the indoor resting area (calf) or the calf creep (cow)	Cow and calf	Instantaneous
Close to the separation barrier	Animal staying within 2 (cow) or 1.5 (calf) metres of the separation barrier	Cow and calf	Instantaneous
Pacing	Calf is moving forth and back parallel to, within 1m of the fence	Calf	Instantaneous

1 Only vocalizations for which the mouth of the cow or calf could be visualized were recorded.

Statistical analysis

For continuously recorded behaviours, values were calculated as frequency of recordings per day. For instantaneously recorded behaviours, values were calculated as the percentage of total observations of which the behaviour occurred. In order to reduce the number of comparisons, we used the median daily frequency (continuously recorded behaviours) or the median daily percentage (instantaneously recorded behaviours) of the behaviour to calculate area under the curve (AUC):

AUC behaviour= [(behaviour day 0+ behaviour day 1)/2]*1+ [(behaviour day 1+behaviour day 2)/2]*1+ [(behaviour day 2+behaviour day 3)/2]*1+ [(behaviour day 3+ behaviour day 4)/2]*1

Two samples Wilcoxon test was applied to test for potential differences between the two separation treatments for all stress behaviours separately. Comparisons were performed two tailed and differences considered significant for p-values below the level of 5%.

FL-separation was performed on 6 bull and 2 heifer calves, while SW-separation was performed on 2 bull and 6 heifer calves. To evaluate if the sex imbalance affected the behavioural measures, we compared each post-separation behavioural response for heifer calves to that of bull calves.

Paper IV – Radiography

Animals and management

To collect data for Paper IV, six Norwegian Red calves (three heifers and three bulls) were borrowed from a dairy farm south of Oslo, Norway. The calves were housed in a group pen (4 by 4 meters) at the Norwegian University of Life Sciences (NMBU) – campus Adamstuen. The animals had free access to water, hay and concentrates. The calves were fed according to the recommended Norwegian feeding regime at the time (Hansen et al., 2011) and received two litres of warm whole milk three times per day at 08.00, 13.00 and 18.00. On three test days their usual morning meal was replaced by milk containing a contrast agent and the animals were radiographed before, during and immediately after the meal. At the first day of the experiment the animals were 19-23 days of age and weighed between 39.5 and 48.5 kilograms.

Experimental design

To enhance the radiographs of the gastrointestinal tract, milk mixed with barium sulphate ($BaSO_4$) (Bracco, Mixobar® Colon (1g/ml)) at a ratio of 6:1, respectively, was fed to the calves. The milk barium:sulphate ratio was determined through a pilot study and was readily accepted by the calf. Lateral-lateral abdominal computed radiography (Kodak DirectView CR 850) with a focus film distance (FFD) of 140 cm using a grid (potter bucky) on standing calves was performed. Exposure factors were 90kV and 32 mAs.

A second pilot study was conducted to determine if any potential milk in the rumen could be discerned from the milk in the rumen. One decilitre barium sulphate solution was administered to the rumen through an oesophageal tube. Immediately after, the calf was allowed free intake of warm whole milk through a teat with a small opening, as with the other calves. The radiographs showed that the abomasum and rumen could easily be distinguished after intake of four litres of milk.

A radiograph from the experiment with annotations is shown below (Figure 5).
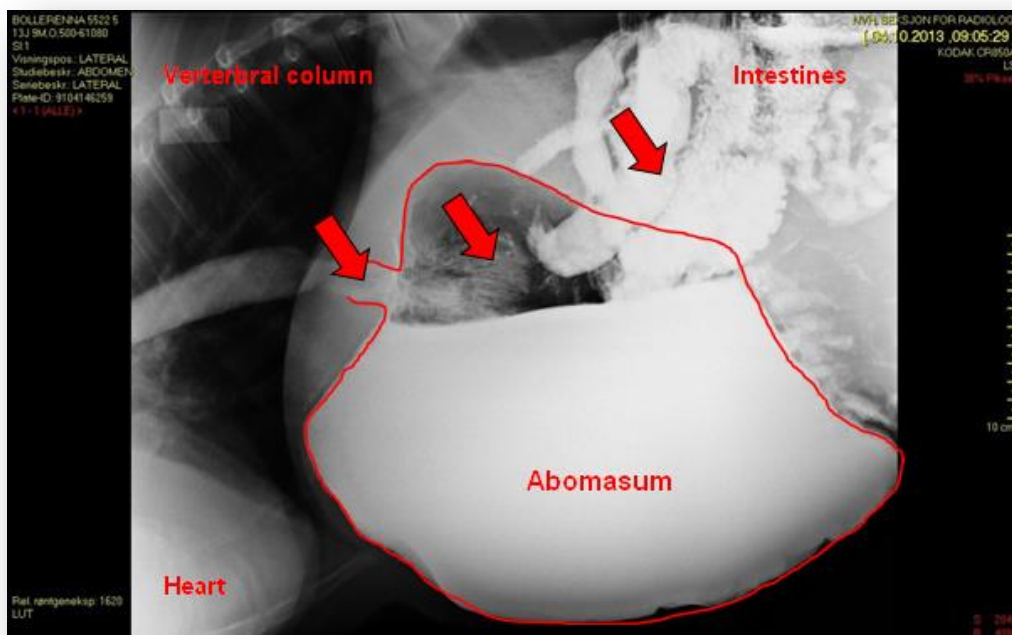
Figure 5. Radiograph of the calf abdomen after drinking 4 litres of milk containing barium sulphate ($BaSO_4$). The vertebral column and heart are located in the upper and lower left corners, respectively. The abomasum is outlined in red. The white in the abomasum is the milk, while the black is gas. The left arrow shows the oesophageal groove and the middle arrow shows the milk squirting into the abomasum. The right arrow shows the intestines with residue contrast agent remaining from the previous test day. The rumen is not visible in the radiograph; hence there is no milk in the rumen. The radiograph has been reproduced with the kind permission of Nina Ottesen (NMBU).

Milk was given from a teat bottle with a relative small opening, allowing a drinking speed of approximately 1.5 litres/minute. The quantity of milk each calf was offered in the test was calculated according to the Response Surface Pathway (RSP) design (Figure 6).

Response Surface Pathway (RSP)

The study was performed as an open, non-randomized and single centre trial with a within patient 3-level Response Surface Pathway (RSP) design (Dewi et al., 2014b). The design consisted of three dose levels. All the calves were started on the same dose (m) at the first design level, but participated in the study based only on their own intake or potential milk in the rumen (Figure 6). The dose for the next level was dependent on the initial dose (m) and the dose adjustment factor k and given by the formula $m_i = m_{i-1} \pm \dfrac{m}{k^{i-1}}$. The upper dose level ($D_U$) is expressed by a finite geometrical series. In this study with three design levels gives $D_U = \dfrac{m(k^n - 1)}{(k^n - k^{n-1})}$. With a starting dose of m = 4.0 litres and an upper dose of D_U = 7.0 litres, gives a k-adjustment factor of k = 2.0.
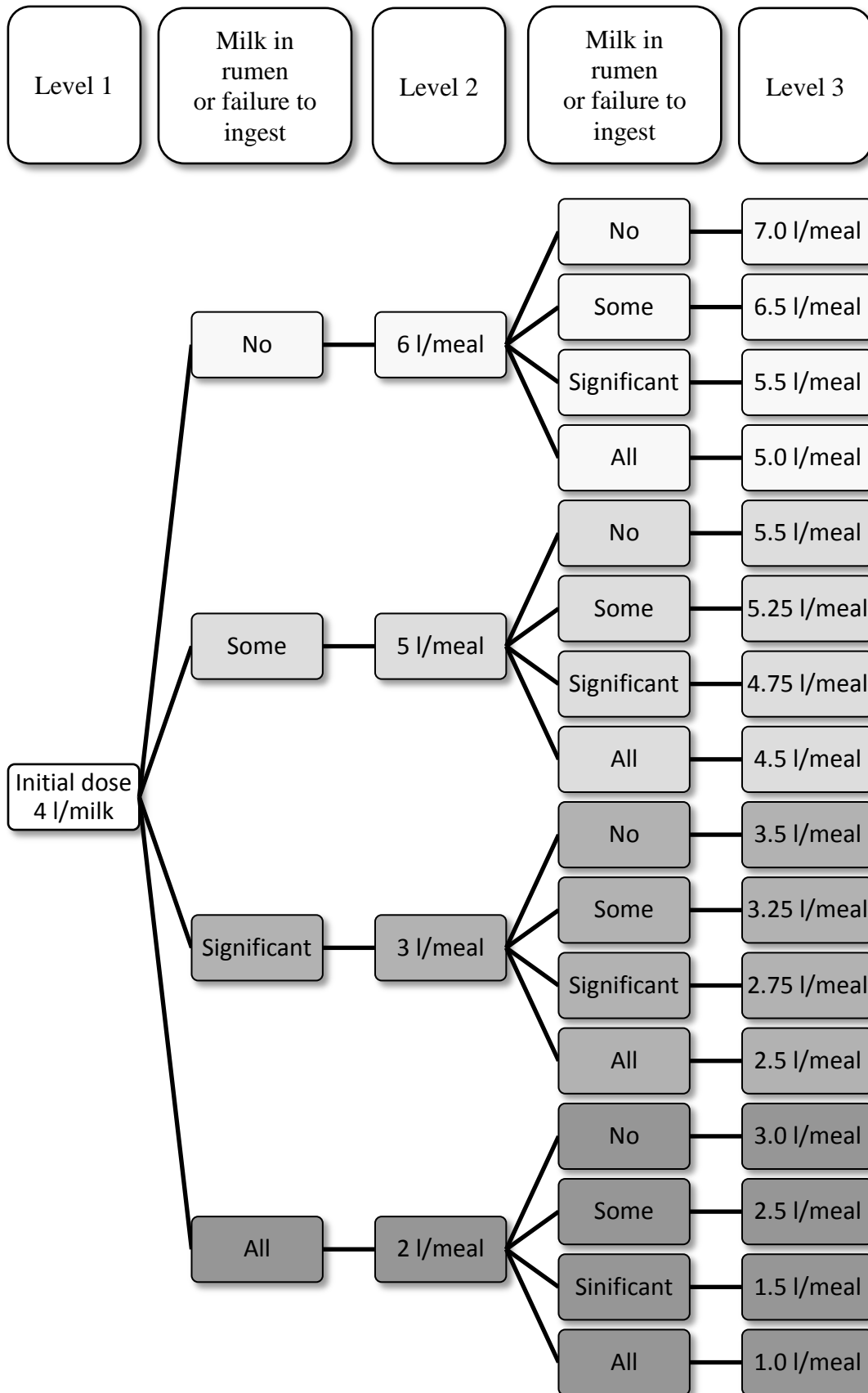
Figure 6. Response surface pathway design with categorized adverse events and cumulative dose in litres milk/meal. Figure by Kristian Ellingsen-Dalskau.

Behavioural observations

Immediately following each of the three test sessions, continuous, live observations were performed in situ to detect behavioural signs of abdominal pain or discomfort in the test animals, indicating reduced welfare. Each observation period lasted for two hours and was performed according to the ethogram below (Table 3). The behaviours included in the study were based on pain behaviours described by Bourne (2013) and clinical veterinary experience.

All behavioural observations were carried out by the author of this thesis, an ethologist and researcher with experience from behavioural observations and pain assessment in animals. The behaviours were observed and recorded on a data logging sheet for each calf individually. Due to lack of personnel, the observer was not blinded to the performance of the calves during trials.

Table 3. Ethogram used to detect signs of abdominal pain or discomfort. All calves were observed for two hours after intake of high amounts of milk and observations were made for each calf individually.

Behaviour	Description	Registration
General impression every 30 min	Normal or dull, (passive, unresponsive and uninterested in surroundings)	Normal (N) or dull (D)
Vocalization/bellowing	High-pitched vocalization	Number of vocalizations per animal
Licking at the stomach region	The animal turns its head and licks at the stomach region	Number of licks per animal
Biting at the stomach region	The animal turns its head and bites at the stomach region	Number of bites per animal
Kicking at the stomach region	The animals turns its head and kicks at the stomach region	Number of kicks per animal
Getting up/lying down	The animal partly or fully stands up or lies down	The number of times calf partly of fully stood up or laid down
Rapid, shallow breathing	The animal shows a bout of rapid, shallow breathing	Number of bouts and duration per bout (sec)
Bruxism	The animal shows a bout of grinding teeth	Number of bouts and duration per bout (sec)
Hunched stance	The animal shows a bout of standing with its head low and back arched	Number of bouts and duration per bout (sec)

It was also recorded whether the behaviours were repeated in a sequence or not. In addition to observing behaviours indicative of abdominal discomfort or pain, potential diarrhoea (defied as faeces with liquid consistency) was recorded for each calf individually.

49

Statistical analysis

Assumed continuously distributed variables were expressed by mean values with 95% confidence interval constructed by the Student procedure (Altman, 1990). Categorized or discontinuously distributed variable were expressed in contingency tables (Agresti and Franklin, 2012).

All comparisons were performed two tailed and differences considered significant for p-values below the level of 5%. Comparisons of continuously distributed variables between design levels were performed using Analysis of Variance (ANOVA) (Altman, 1990). Additionally, isotonic regression analysis for multinomial outcome was performed (Paul et al., 2004; Dewi et al., 2014b).

Ethical considerations

In Paper I the anonymity of the respondents was ensured through the use of QuestBack™ as QuestBack by default hide the identity of the respondent. Also, all responses were pooled for the statistical analyses, hence preventing identification of single participants.

It is well-known that test subjects, being animals or humans, alter their behaviour when they know they are being observed. This is often referred to as the observer effect or Hawthorne effect (Martin and Bateson, 2007). In an attempt to obtain "true" stockperson behaviour, the stockpeople in Paper II were blind to the real purpose of the study. After the test had ended, each stockperson was informed that the true objective of the study was to record their behaviour, not to record the chest measurement of the calves, At this point, all 110 participants were offered the possibility of withdrawing from the experiment, but all consented to participation under the prerequisite that all results were randomized and their anonymity maintained.

Papers II, III and IV directly involved the use of animals. Although non-invasive, these trials are therefore regarded as animal experiments. Due to the nature of the experiments, obtaining permission from the Norwegian Animal Research Authority was not required. Nevertheless, Kristian Ellingsen-Dalskau passed a FELASA Cat C course before the experiments were initiated.

All procedures were in accordance with the regulations controlling experiments/procedures in live animals in Norway, and the studies comply with the policies relating to animal ethics. Animal health was monitored daily. The calves in papers II and III were tested on their respective home farms, and all calves in paper IV were returned home after the experiment.

Main results

Paper I

Results from the questionnaire showed that low calf mortality and adequate treatment when animals were ill or injured were rated most favourably for calves in organic dairy production. Body condition and growth received the least favourable scores.

Both veterinarians and agricultural advisors considered overall health and well-being to be equal for calves in organic and conventional dairy farming systems. However, some differences were discovered when comparing the two systems. Calves' confidence in people and space allowance was considered to be better in organic dairy herds, both by the veterinarians ($p = 0.002$), and by the advisors ($p = 0.022$). Feed quality in organic production, was regarded as poorer by both professions ($p = 0.001$ for the veterinarians, $p < 0.001$ for the advisors). When considering organic farming alone, the routine of keeping calves with their mothers was seen as the most important welfare advantages (45% of the veterinarians and 30% of the advisors). The second most important welfare advantage was good calf care/stockmanship (14% of the veterinarians and 21% of the advisors). Both veterinarians and advisors pointed to poor feed as the greatest welfare challenge for calves in organic farming systems (22% of the veterinarians and 39% of the advisors). While the veterinarians considered inadequate disease treatment to be the second most important welfare challenge (19%), the agricultural advisors pointed to poor hygiene concerns (26%).

Results from the questionnaire indicated that the participants' familiarity with organic standards was relatively poor, especially among the less experienced professionals. Advisors, regardless of experience, found themselves to be more familiar with the regulations concerning organic dairy cattle husbandry than did the veterinarians ($p < 0.001$), but no differences were found concerning medicine use.

Paper II

Principal component analysis classified calf behaviour along two axes labelled positive/negative mood and high/low arousal. The two components explained 45.4% and 15.7% of the variance, respectively. Using the same method on the stockperson behaviour, four handling styles were discovered: (1) calm/patient, (2) dominating/aggressive, (3) positive interactions and (4) insecure/nervous. The four components explained 25.5%, 24.4%, 18.1%

and 16.9% of the variance, respectively. PC1 and PC3 can be viewed as positive, PC2 and PC4 as negative.

Using structural equation modelling, it was found that stockpeople who actively talk to and pet their calves (β = -0.41), as well as those who are calm and patient when interacting with their animals (β = -0.28), have calves with lower levels of negative mood, as characterized by high scores on QBA items like friendly and content. Stockpersons with a nervous handling style (β = 0.25), or who were dominating and aggressive (β = 0.23), on the other hand, had calves with higher levels of negative mood.

Paper III

Results showed that cows and calves in both treatments were highly reactive during the first days after separation. Nevertheless, some treatment differences were discovered for the calves. Calves separated by FL-separation performed less high-pitched (open mouthed) vocalizations compared to SW-separated calves (p = 0.003).

Calves separated with FL also showed a lower frequency of alert behaviour than SW calves (p = 0.003). Alert behaviour was higher for SW throughout the observation period, while FL calves displayed a rapid decline after day 0. All calves except two (one from each treatment) were observed to put their head through the fence at day 0. However, there were large individual differences and no effect of treatment.

The post-separation behaviours of cows were not statistically different between the FL and SW treatments. All cows except two, one from each treatment, were observed to put their head out of the pen. All cows spent some time close to the separation barrier, and for both treatments this behaviour varied greatly both individually and from day to day. Alert behaviour was shown by all cows during the first 3 days, and whereas most SW cows continued to show alert behaviour throughout the trial, only a few FL cows were occasionally observed performing this behaviour beyond day 2.

Paper IV

Fed unrestricted portions of warm whole milk from a teat bottle, the calves drank high volumes of milk. Four out of the six calves drank five litres or more in one meal, while two of the six calves drank more than six litres when the highest amounts were offered. The highest voluntary intake was 6.8 litres in one meal (13.2% of BW). Abdominal radiographs showed

that the abomasum has great capacity for expansion. Despite the fact that the calves on several occasions exceeded the recommended daily volume in one meal, no milk in the rumen or abdominal pain or discomfort was observed in any of the calves, regardless of intake.

Discussion

Methodological considerations

Paper I – Measuring perception

Perception and objectivity

The focus of Paper I is the health and welfare of calves, especially in organic production. The data is based on a questionnaire asking for the *opinions* of veterinarians and agricultural advisors linked to this production. Hence, the results are based on perception of current status rather than actual status measured on the farm. Perceptions are always influenced by attitudes, and attitudes again are influenced by innumerable factors, including gender, education, and experiences (Eagly and Chaiken, 1993; Ellingsen et al., 2010). Potentially differing opinions, however, was one of the things we sought in Paper I.

Pooling the opinions of the veterinarians with those of agricultural advisors was also a way of making the data more representative and reducing any potential bias. The veterinarians and agricultural advisors were also heavily experienced, indicating that they are qualified for evaluating the conditions on the farms.

The use of electronic questionnaires as a research tool exploded during the 1990s, due to obvious advantages, especially for a targeted population (Shannon et al., 2002). Questionnaires developed and distributed electronically are inexpensive and are also fast to respond to (Mavis and Brocato, 1998). Data analysis is also generally easy, as online research tools are compatible with spread sheet or statistical analysis software programs (Shannon et al., 2002). Receiving an electronic invitation to participate in a survey also means that potential respondents can reply how and when they feel comfortable (Selwyn and Robson, 1998), as opposed to direct or phone interviews. Electronic surveys are also easy to follow-up, as reminders can be sent to non-responders at predefined times. Problems regarding social desirability may arise, but incidences are lower in anonymous internet-based questionnaires compared to non-anonymous paper-based methods (Joinson, 1999). Due to our targeted population, as well as the practical and economic advantages, electronic questionnaires were chosen for data collection in Paper I.

Uni- and bivariable non-parametric statistics

The data in Paper I was analysed using only uni- and bivariable statistical methods. Univariable statistics are the simplest form of quantitative analysis and implies that only one explanatory variable is tested at a time without taking other variables or confounders into account (Kachigan, 1986). Examples of univariable analyses are e.g. frequency distributions, histograms and mean scores. Univariable analyses are commonly used in the descriptive stages of research, which was also the aim of Paper I.

The next level of statistical complexity is bivariable analysis, in which two variables and the relationship between them are investigated. Examples of bivariable analyses are Wilcoxon rank-sum tests, Wilcoxon signed-rank test and Pearson's chi-squared test (Gibbons, 1993), all performed in Paper I. These non-parametric tests were chosen because the data in Paper I was not normally distributed. Because non-parametric methods make fewer assumptions, they are more robust and may be simpler to use. The disadvantage, on the other hand, is that non-parametric tests have less power, and a larger sample size can be required to draw conclusions with the same statistical confidence (Gibbons, 1993).

In real life, variables do usually not exist as isolated items, but function as part of an integrated system. Efforts, therefore, should be made to apply multivariable statistical methods like regression analysis. However, the relatively low number of replies prevented us from doing so.

Paper II – Qualitative Behaviour Assessment

The validity of QBA for animal welfare assessment

After the data for Paper II was collected, three articles have been published that may question the validity of QBA. In the first study, Stockman et al. (2012) failed to find significant correlations between QBA scores and temperament measures in beef cattle at slaughter. In the second study, large and inconsistent variation in the inter- and intra-observer reliability was found among experienced and inexperienced observers (Bokkers et al., 2012). In the third study, QBA scores were compared with the cattle welfare protocol from Welfare Quality® (WQ), and no significant pattern of relationship was found (Andreasen et al., 2013). A number of potential explanations for the poor relationship between QBA and other instruments were proposed. For instance, in the WQ article, it was suggested that the different outcomes were due to the fact that the WQ protocols are not purely animal based, that QBA is designed to pick up small variations in behaviour whereas the WQ protocols are not, and that

there were not enough variation in the QBA scores (Andreasen et al., 2013). It is also important to note that many of the poor associations among observers found by Bokkers et al. (2012) were based on the individual descriptors, not the QBA dimension. Although recognizing the fact that dimension reliability is based on the individual descriptors, Wemelsfelder stresses that it is the QBA dimensions (after performing the principal component analysis) that are relevant for animal welfare, not the individual terms (Wemelsfelder and Millard, 2009; Wemelsfelder, pers. comm).

Even so, the weak associations found between QBA and other indicators likely point to some of the challenges of QBA. Bokkers et al. (2012) failed to find important differences between experienced and inexperienced observers, and claim that observers should make the same observations irrespective of background. This is a valid argument, but, as the study was done using fixed terms, observers may not necessarily infer the same meaning into the pre-defined terms. Weighing the observations of different animals on-farm may also pose a challenge. The descriptors are rated as a mean impression of all observed animals. How one or two animals in severe pain are weighed against the rest of a healthy herd needs to be thoroughly discussed. Animals kept on pastures can also be difficult to approach, making observation hard. A large number of animals in one place can also lead to different observers catching the behaviour of different animals, hence reaching dissimilar conclusions about the unit. A further challenge can be that production units can give different impressions due to recent activities like moving or mixing of animals, depending on where they are in the production cycle and the time of day (Gutmann, et al., 2015). These points all highlight the need for observers to sit together and agree on the scoring of the descriptors, either on-farm or by video. The importance of training is also emphasised by Wemelsfelder, (2009c), as well as periodical calibration to avoid drifting (Bokkers et al., 2012). As QBA is designed to identify small variations in behaviour, observers should know the target species and the species-specific behaviour (Wemelsfelder, 2007). Observers also need experience in observing animals in different contexts, and a willingness to communicate with animals as sentient beings (Wemelsfelder, 2007).

Regarding the statistical analysis, QBA with fixed terms is analysed with principal component analysis (PCA), which cannot be calculated by hand, but rather requires some sort of statistical software. PCA also requires at least one production unit per QBA item (Jolliffe, 2002). This means that for QBA to be efficiently analysed on-farm, software for e.g. a table PC needs to be developed containing a pre-made database with QBA data, which again may reduce feasibility.

Much of the validation performed on QBA has been done on the free-choice profiling (FCP) approach rather than on fixed terms. In Wemelsfelder et al. (2001) and Wemelsfelder and Lawrence (2001), FCP is favoured because it allows independence of the observers, while pre-defined terms restrict the descriptors' options, obstructing the integrative character of qualitative assessment. Even so, FCP is unsuitable for on-farm assessment because it is complex, time-consuming and requires at least 10 observers for statistical purposes (Wemelsfelder and Millard, 2009). To accommodate for this, development and validation of fixed lists of qualitative descriptors for on-farm use were investigated in the Welfare Quality® project, with relative success. The correlation between FCP and fixed terms has also been scrutinized in a master thesis from 2011 (Müllner, 2011). Müllner found high inter-observer reliability for both the FCP- and FT-approaches, and highly significant correlations between the two approaches.

Despite its challenges and need for further refinement, QBA has a number of advantages. One of the major advantages is that judgement is based on the entire animal. Rather than choosing an isolated focus area like cleanliness or lameness, QBA takes the whole animal and its appearance into consideration when evaluating the level of welfare. This also avoids the problem of integrating different aspects of welfare, e.g. animal- and resource-based indicators, into a single measure (Andreasen et al., 2013).

Quantitative methods may also omit potentially important information as they are not designed to identify subtle behavioural variations, behaviours that occur with low incidence or behaviours that are difficult to quantify (Rousing and Wemelsfelder, 2006). QBA also appears to have high face- and construct-validity, because it allows us to predict problems of animal welfare in daily life based on animal communication (Wemelsfelder and Lawrence, 2001). Face validity is the extent to which a test appears to measure the construct of interest, while construct-validity is the extent to which it actually succeeds in measuring what it claims to measure (Price, 2012).

QBA is suitable for animal welfare assessment due to its ability to capture the affective state of animals. Animal welfare is concerned with the quality of the relationship between the animal and its environment. To assess this quality, we need to integrate everything we perceive and the context in which we perceive it (Wemelsfelder, 2007). Hence, the body language of animals is more than mechanical movement. It contains a psychological dimension that is open to scientific analysis (Wemelsfelder, 1997), allowing us to directly assess animal welfare.

As discussed above, QBA has a number of practical and technical challenges that need to be overcome. Nevertheless, due to its ability to capture the subjective and both negative and positive mental states of animals, QBA was the method of choice for Paper II.

Study limitation

A limitation to Paper II is the fact that QBA1 (calves and young stock) and QBA2 (stockpeople) were carried out by the same person. Having the same observer performing the two QBAs could cause observer bias. For instance, if the observer recorded mainly negative mood among the calves, he might be more prone to scoring the stockperson higher on descriptors like "aggressive" and "boisterous" and vice versa. This limitation means that the animal and stockperson QBA scores are not independent.

The total time spent observing the animals should have been standardised to 20 minutes, divided on the number of observation points. Also, the QBAs should have been performed with the same time interval since last feeding on all farms. By doing so, animal behaviour would not be influenced by differing levels of satiation or hunger.

To ensure the independence of the two QBAs, two observers should have been involved in the observations. When performing the QBAs, the first observer should have completed QBA1, while the second observer remained outside the barn. After QBA1 was finished, the second observer should have performed QBA2 without consulting the first observer. Testing of repeatability should also have been performed, e.g. using video clips. The use of two observers visiting all farms was not practically feasible for economic reasons. One possible way of including a second observer without greatly increasing the expenses, would have been to video tape the QBA sessions for retrospective scoring by a second observer. However, due to personal information protection concerns, only QBA1 could have been videotaped.

No rotation of the PCA axes

As previously explained, QBA is generally analysed with PCA analysis. A common way to optimise factor loadings when performing PCA is to rotate the axes, using either Varimax or Oblimin rotation depending on the level of correlation among the included items (Jolliffe, 2002). In the analysis of QBA data rotation is not common. Rotation of the axis disturbs the interpretation and Wemelsfelder says she does not rotate to stay as close as possible to the actual data to avoid distrust (Wemelsfelder, pers. comm).

To analyse the stockperson QBA Varimax rotation was performed to make the handling styles as clear as possible.

Structural equation modelling (SEM)

SEM does not provide any more evidence of causality than does regression. However, as the model is built on a previously established expected relationship, the model can, to some degree, be said to be causal. Also, SEM does provide evidence of goodness of fit to a causal model. One must therefore decide, for each measure of fit, what represents a sufficiently good fit between the model and the data. Here, other contextual factors such as sample size and complexity of the model must also be taken into consideration (Byrne, 2010).

An important part of animal welfare research, as with all research, is to clearly communicate the results. This is an advantage of SEM, as also used in Paper II. Being able to physically draw the variables and how they relate to each other makes SEM a graphic, honest and easy-to-understand way of communicating results (Byrne, 2010).

Paper III – Area under the curve and nonparametric testing

Methodological issues

Some challenges arose when conducting the experiment for Paper III. A relatively low number of animals, 16 cow-calf pairs, were included in the study. When analysing the data, great variations in frequency were discovered and the data was not normally distributed. For these reasons the data had to be analysed using a non-parametric test (Gibbons, 1993). There was also a sex imbalance among the test animals. Six bull calves and two heifer calves were separated by SW, while two bull calves and six heifer calves were separated by FL. Statistical analyses were performed to see whether this had any effect on our results and conclusions.

The animals were observed for four hours (divided on two 2-hour observation bouts) per day at days 0-4. When observing animals, the main concern is to observe at times representative of the behaviour of interest (Martin and Bateson, 2007). Therefore, pilot studies were performed to find the optimal time for observation. As a result, the most active periods were chosen to have the greatest chance of picking up the reinstatement behaviours. Ideally the animals could have been observed continuously for 24 hours per day during the observation period, but this would have been too resource demanding and was not feasible.

A potential limitation to the study is that all calves were separated in pairs. Calves form strong bonds with their peers (Bøe and Færevik, 2003) and this social contact may have

reduced the post-separation stress responses (Duve et al., 2012). Individual housing of calves beyond one week is prohibited in organic dairy production (Mattilsynet, 2015).

No inter-observer reliability analyses (Martin and Bateson, 2007) were performed to check the degree to which the three observers agreed on the behaviours they observed. However, all observers were trained and synchronised for the task through discussions and pilot projects. The short observation bouts also helped avoiding observer fatigue (Martin and Bateson, 2007).

Statistical analysis

Area under the curve (AUC) was chosen as the initial step in the analysis of Paper III. AUC is a transformation technique that generates a new outcome variable based on summation of frequencies for the full test period. Hence, the AUC for a specific behaviour resulting from either FL or SW treatment for days 0-4 was calculated. Subsequently, potential treatment differences between the new outcome variables were tested for by using two samples Wilcoxon tests. AUC allowed us to test for effect of treatment only. AUC is a robust method, but has lower statistical power than parametric methods

If more animals had been included in the study and the data had been normally distributed, the data could have been analysed using Mixed-model design ANOVA (Pallant, 2010). This is an analysis in which two types of variables are involved, at least one between-subjects variable and at least one within-subjects variable. The between-subjects variable would have been separation method (FL or SW) and the within-subjects variable would have been day since separation (0-4). This would have allowed us to investigate the main effects of treatment and day, as well as the treatment-day interaction effect.

Paper IV – Response surface pathway

Why RSP?

When doing research on animals, we are obligated to implement the 3R's (replacement, reduction, refinement). Using as few animals as necessary to provide sound science is an advantage with the Response Surface Pathway design used in Paper IV. By allowing each animal to act as its own control, the number of animals required to achieve the same statistical power as with an equivalent method, is reduced to a minimum (Dewi et al., 2014a; Dewi et al., 2014b).

In Paper IV, the RSP design was chosen as the method to determine how much milk each calf would receive on each level. RSP was originally developed to reduce the number of laboratory animals needed to estimate toxic levels and dose-response curves, without the loss of information (Dewi et al., 2014b). The method can also be applied to other areas. An advantage with the RSP design is that it does not require any prior probability distribution. The results obtained on one level dictate the dose to be used on the next (Dewi et al., 2014b).

Study limitations

Between- and within-patient RSP designs are generally analysed with isotonic regression analysis (Dewi et al., 2014a; Dewi et al., 2014b). An advantage of isotonic regression is that it does not assume any form for the target function, such as linearity assumed by linear regression (Salanti and Ulm, 2005). In paper IV it turned out that isotonic regression analysis could not be utilised. Voluntary intake of milk up to 6.8 litres was insufficient to cause milk to enter the rumen. This means that there were no quantiles to estimate and hence isotonic regression could not be applied to determine an optimal dose level based on abomasal capacity. This limitation can be attributed to the design and is not a problem with isotonic regression per se.

Nevertheless, not being able to use isotonic regression does not weaken the results. Due to the firm belief that calves should not be fed milk meal sizes exceeding 2-3 litres, we expected that free voluntary intake would be sufficient to cause milk to enter the rumen. Also, as we proved that such high volumes of warm whole milk drunk from a teat bottle is safe to give, we can still recommend that farmers increase the amount of milk they feed their calves, far beyond what is practically necessary.

Alternative design

If we had known that 6.8 litres of milk was insufficient to cause milk to enter the rumen, an alternative design could be used. One such design is the Up-and-down procedure, first described in 1948 (Dixon and Mood, 1948). There are several ways to apply this method, but one way consists of a single ordered dose progression in which animals are dosed one at a time (United States Environmental Protection Agency, 2010). In our case, the first test animal could be given four litres of milk. If no milk in the rumen was detected after intake, the amount of milk for the next animal would be increased to a factor of one half log times the original dose. If milk in the rumen was detected, the amount of milk for the next animal

would be decreased by the same amount. Dosing is stopped when one of the pre-defined stopping criteria is met and the optimal meal size and confidence intervals (CI) are calculated based on the status of all the animals at termination using maximum likelihood method (United States Environmental Protection Agency, 2010).

Study results

Animal welfare in organic dairy production (Paper I)

Conflicts between regulations and practices

Organic farming has regulations which should allow for better animal welfare. Still, conflicts may arise between regulations and the wish to secure high levels of animal welfare. One such issue is regarding feed quality. Results from Paper I showed that poor feed quality was viewed as the most important welfare challenge in organic farming by veterinarians and agricultural advisors alike. Poor feed quality was also the only factor considered being inferior in direct comparison to that of conventional dairy production. Poor ratings on body condition and growth among organically produced diary calves also found in Paper I further support this concern.

A likely explanation for this matter is the requirement that at least 50% of the feed has to be produced on the farm or in cooperation with other organic producers (Mattilsynet, 2015). Providing animals with highly nutritional organic feed can prove difficult. This is especially true for a country like Norway where dairy farms often are located in mountainous areas with poor growth conditions. However, providing adequate organic feed for cattle has also been identified as a problem in Denmark (Christiansen and Boesen, 2001).

A second conflict between regulations and the wish to secure good animal welfare is related to disease treatment. In Paper I veterinarians rated inadequate treatment of disease as the most important welfare challenge for calves in organic dairy production after poor feed. Except for vaccines and anthelmintic drugs, as well as treatments ordered by the authorities, routine or prophylactic use of chemical or synthetic drugs in organic farming is prohibited (Mattilsynet, 2015). Disease treatment in organic livestock production is based on the assumption that feeding, housing and animal care provides an optimal natural resistance to fight disease. According to a review article by Kijlstra and Eijck (2006), this has not yet been scientifically proven. There is also a solicitation to use alternative disease treatment including homeopathy and plant extracts. A PhD in homeopathy was passed in Norway in 2004, but

failed to find any significant treatment effects (Hektoen, 2004). Also, Swedish veterinarians are prohibited from treating animals with homeopathic remedies. The use of non-evidence based alternative medicine may result in suboptimal disease treatment and reduced animal welfare.

Are veterinarians more sceptical towards organic production?

Veterinarians were among the first to express concern about animal welfare in organic production (Vaarst et al., 2001). Still, veterinarians have often been criticized for having little knowledge about the organic standards and welfare philosophy (Vaarst, 1998). One of the aims of Paper I was therefore to investigate whether veterinarians were more sceptical against the animal welfare status in organic diary production than other professions, in this case agricultural advisors. Overall, the veterinarians and the advisors differed very little in their evaluations. Some differences were discovered, but they are more likely to reflect differences in emphasis than actual differences. The claim that veterinarians are more sceptical towards organic production is therefore not supported. In a Danish interview survey among practicing veterinarians and advisors, Vaarst (1998) found that the veterinarians expressed this type of concern (e.g. that organic animals were not treated properly), but when in-depth interviewed about concrete cases, none of them could give actual examples based on their own experience. The need for good advisors and veterinarians educated in the holistic approach of organic livestock farming and preventive disease management was identified as a key area after the three year EU network project Sustaining Animal Health and Food Safety in Organic Farming (SAFO) (Vaarst et al., 2008).

It is also important to keep in mind that scepticism could be the result of the time the respondents were educated. The veterinarians and advisors included in our study were highly experienced making them apt to evaluate the production system. On the other hand, it also means that the respondents were educated at a time when animal welfare was mainly judged on basis of biological functioning. Presumably there will be changes in attitude and the level of knowledge in the years to come with newly educated veterinarians and agricultural advisors and an increasing number of organic farms.

The three day suckling rule

The three day suckling rule states that the calf must be allowed to suckle its mother for at least three days after birth (Mattilsynet, 2015). In Paper I, this procedure was viewed as the greatest advantage in organic dairy farming. The rule is nevertheless highly controversial. The

main arguments against keeping calf and cow together are economical (more milk can be sold to the dairy if the calf is fed milk replacer), control of milk intake, and stressful separation for the animals involved (e.g. Flower and Weary, 2003; Stěhulová et al., 2008). On the other hand, a number of studies have also showed that allowing the cow and calf to stay together results in health benefits like increased weight gain and reduced occurrence of diarrhoea (Krohn, 2001; Flower and Weary, 2003).

Suckling in dairy production has been the topic of a recent doctoral thesis in Norway (Johnsen, 2015). In her thesis, Johnsen comes up with several, alternative and animal-friendly ways of allowing cow and calf to stay together while still producing milk. It is also important to recognise organic producers as pioneers within the dairy industry. Perhaps extending the suckling period rather than reducing it would cause others to follow?

So, better animal welfare?

In summary, organic production has several prerequisites which should allow for better animal welfare than conventional production. The organic philosophy has a strong emphasis on natural behaviour and the organic legislation generally has higher levels of minimal standards. However, results from Paper I showed that overall health and welfare was viewed as equally good for calves in organic and conventional dairy production in Norway (Ellingsen et al., 2012). Also international studies fail to find noteworthy differences in animal welfare between the two production systems, e.g. as shown in the review articles by Sundrum (2001), Lund and Algers (2003) and Kijlstra and Eijck (2006). The results from Paper I indicate that organic dairy farming does not provide better animal welfare compared to conventional dairy farming. This indicates that the implementation of the legislation, the management practices and the personal motivation of the farmer has greater impact on the level of animal welfare.

Although differences are currently small, it is important to keep in mind that organic animal husbandry is relatively new. The system is still under development and research on the topic has so far been relatively scarce. It is therefore important to recognise the potential of these systems, as the organic standards have a substantial welfare perspective (Lund, 2006).

Independent of production system, there is frequent contact between the stockpeople and intensively raised animals like dairy cattle. Interactions often occur during routine husbandry procedures like artificial inseminations and pregnancy controls, cleaning and milking (if not AMS). Although many of these procedures enhance welfare in the long run,

they are perceived as negative by the animals (Raussi, 2003). Good stockmanship and a good HAR is therefore essential for good animal welfare.

The importance of good stockmanship (Paper II)

Stockmanship, welfare, health and production

The daily interaction between the stockperson and his/her animals determines the quality of the HAR (Hemsworth et al., 1981a; Hemsworth et al., 1981b). Results from Paper II showed that stockpeople who handled their calves in a positive way had calves with higher levels of positive behaviour and mood. Stockpeople who exerted a negative handling style, on the other hand, had calves with higher levels of negative behaviour and mood. The idea of farmer and animal as a double mirror, observing and reflecting each other's behaviours is also discussed by Biovin et al (2000). In Paper I stockpeople taking good care of their calves was also rated as the second most important welfare advantage in organic dairy production.

The effects of negative handling on welfare and health in animals have been widely documented. A large number of publications have found a relationship between negative handling with hitting and shouting and fear in animals (Rushen et al., 1999a; Rushen et al., 1999b; Pajor et al., 2000; Munksgaard et al., 2001; Rushen et al., 2001; Hemsworth, 2003; Raussi, 2003). Fear leads to acute and chronic stress and has a profound negative effect on animal health and welfare (Grandin, 2010). On the other hand, the positive effects of good handling have also been documented. Farms with stockpeople with positive attitudes and a positive contact with the calves have been associated with lower levels of disease and lower mortality rates (Lensink et al., 2001b). Heifers receiving positive treatment by humans with stroking and brushing were also found to voluntarily re-approach and interact with the human after the human had retreated (Bertenshaw and Rowlinson, 2008). Bertenshaw and Rowlinson suggest that positive treatment constitutes an environmental enrichment of dairy cattle and improves their quality of life.

Closely linked to animal welfare and health is animal production. Studies on dairy cattle have shown that farms where cows are most frightened of people have lower milk yield and cows withholding up to 70 % of the milk (Rushen et al., 1999a). Calm touching and positive interactions with the animals, on the other hand, led to increased milk yield (Bertenshaw and Rowlinson, 2015). Seabrook (1984) reported a 13% difference in milk yield between gently and aversively handled cows. In a more recent study, Breuer et al. (2000) reported that 19 % of the variance in milk yield among production units was related to the

degree of fear cattle show towards people. With regards to calves, Lensink et al (2000a; 2000b; 2001b) observed that positive interactions with petting and talking appeared to lead improved production results with enhanced growth rate and meat quality. Similar studies have also been performed on other domestic species with similar results (see Hemsworth and Coleman, 1998 for review). The impact of stockperson behaviour on production was not, however, included in Paper II.

Safe and easy handling

Goode stockpeople were in Paper II found to be people who are calm, patient and cuddle and talk to their animals. Such positive handing is not only beneficial to the animals. Indirectly, it is also beneficial to the stockperson. Animals experiencing a positive HAR are safer and easier to handle (Hemsworth et al., 2000; Hemsworth, 2003; Simensen, 2004). Experiencing negative or little human contact can make the animals hard to approach and result in increased flight distance (Breuer et al., 2003). This can again lead to animals attacking or otherwise hurting themselves or the stockperson (Rushen et al., 1999b). Positive behaviour by the farmer has also been found to reduce calves' emotional and behavioural responses to handling and transport (Lensink et al., 2000c; Lensink et al., 2001a).

In Paper II we suggest that there is a negative feedback loop running from handling style to calf mood back to handling style. For instance if a stockperson is insecure or nervous, his/her cattle may respond with more negative behaviour making them harder to handle. This may again feed back to the stockperson pushing him/her to be more forceful. This feedback loop is also likely to be active for positive handling styles. This was in part confirmed by Lensink et al (2001b) who found that positive attitudes among stockpeople resulted in improved herd health, resulting in a more welfare-friendly environment for the calves.

Other reasons for treating our animals well

In addition to improving animal welfare, health and production and making animal handling easier, there are also other reasons for treating our animals well. Firstly, there is a legal incentive. It is stated in the Animal welfare act that "Animals should be treated well and protected against unnecessary stresses and strains" (Landbruks- og matdepartementet, 2010). Secondly, there is an economic incentive. Healthy animals have high feed conversion ratios, grow well (Irsik et al., 2006), and seldom need medical treatments. Thirdly, we have a moral obligation to care well for the animals in our custody. Lund et al. (2004a) suggest that an ethical contract exists between the farm animals and the humans, ensuring that the created

wealth is shared, that animals are cared well for and the animals are not exploited (Lund et al., 2004a). Fourthly, animals must be treated well to avoid consumer distrust as seen in many other countries (Skarstad et al., 2007).

Shaping attitudes through training

Paper II showed a direct link between handling style and animal welfare. This means that careful selection and proper training of stockpeople is needed for people who are working with animals. It also means that stockpeople need to be self-critical and reflect on the way they act in the presence of animals. Treating animals well sends important signals to the public, but is also important for the internal work culture. Work cultures are different at different production units and attitudes and behaviours are easily spread among the stockpeople (Hemsworth and Coleman, 1998). Several authors list the farm manager's attitudes as the most important factor determining the quality of animal handling (Grandin, 2000). Also, foreign workers with different backgrounds are often employed in the livestock industry. Managers should therefore recognise their responsibility as role models. By promoting positive attitudes and treating the animals well, they set the tone for the rest of the stockpeople.

A good HAR is important to reduce stress and anxiety in animals during stressful events. Maintaining a close relationship with other animals may also have the same effect, for instance during separation.

Physical contact as a way of reducing post-separation stress (Paper III)

Alternative separation methods

Separation, regardless of when it occurs, will inevitably lead to stress and have a negative effect on the welfare of cow and calf (e.g. Weary and Chua, 2000; Stěhulová et al., 2008; Ventura et al., 2013). Paper III showed that allowing cow and calf to have physical contact after separation can alleviate the behavioural response of the calf. Calves receiving the fence-line treatment were found to perform less high-pitched vocalization and showed less alert behaviour than calves receiving the solid wall treatment. For cows no significant differences were found.

Studies utilising fence-line separation frequently report contradictory results. In accordance with Paper III, fence-line separation has been found to reduce the vocal reaction of beef cattle calves compared to abruptly weaned animals, indicating lower stress levels

(Stookey, 1997; Price et al., 2003). The same studies also found fence-line separation to prevent both short and long term weight loss. However, those parameters were not included in Paper III. Other studies have, on the contrary, found physical contact post-separation to have no effect or to be more stressful for the animals (Solano et al., 2007; Enríquez et al., 2010; Enríquez et al., 2011).

Ambiguous results have also been found for two-step weaned calves. While some claim that cows and calves separated in two steps show fewer signs of stress at weaning (Haley, 2006; Loberg et al., 2008), others argue that two-step weaning does not reduce the distress reactions, rather they redistribute them over two periods (Enríquez et al., 2010; Enríquez et al., 2011).

The results from Paper III are based on the behaviour of dairy cattle. Most fence-line and two-step separation research has been performed on beef cattle. This means that both the age of the animals and the management routines at the point of separation are different. This hence complicates the comparisons. Beef cattle are usually weaned by separation, meaning they are both weaned and separated from their mother at the same time at about six months of age. In Paper III, the dairy calves were separated at 8 weeks of age, but continued to receive milk. In this way, Paper III can be said to perform a revers two-step manner where separation occurred prior to weaning. The major difference between the calves in the FL and SW treatments was the physical contact allowed with the dams. The reduced vocalization and alert behaviour seen in the calves are thus likely to result from the physical contact. Staying close to each other is a natural response of cows and calves at weaning independent of whether or not the calf is suckling (Veissier and Le Neindre, 1989).

Using high-pitched vocalization as an indicator of stress

In Paper III it was found that calves separated by FL showed a higher frequency of high-pitched vocalization than SW separated calves, while no differences were found for low-pitched vocalizations. This is the first study to quantitatively assess these two kinds of vocalizations in cattle in response to forced separation. Low pitched vocalizations are often performed as part of the bonding and are generally not associated with stress (Padilla de la Torre et al., 2015). High-pitched vocalization, on the other hand, is performed when calves are unable to locate their mothers (Haley, 2006; Padilla de la Torre et al., 2015). Also according to Weary et al. (2008), high-pitched vocalization is a way of communicating a need, and is probably emitted as an attempt to modify the behaviour of receiver, in this case the dam. The higher frequency of high-pitched vocalizations performed by SW calves in

Paper III is therefore likely to signal an unfulfilled motivation to reunite and higher levels of stress. Quantification of high-pitched vocalization hence seems a promising tool for assessing distress at separation.

The importance of keeping the calf satiated to reduce stress during separation was recognised both by Norwegian and Swedish farmers (Ellingsen et al., 2015) and others (Thomas et al., 2001). Also after separation milk feeding has great impact on calf health and welfare.

Milk feeding in dairy calves (Paper IV)

Capacity of the abomasum

In Paper IV abdominal radiographs showed that the abomasum has great potential for expansion. Although the test animals were allowed unrestricted portions of milk and several calves reached meal sizes exceeding five and six litres, the high volumes were insufficient to cause milk to enter the rumen. The fact that the voluntary meal size on several occasions exceeded the daily recommended intake also showed that the calves desire higher volumes of milk than they normally receive. Until recently, the Norwegian milk feeding recommendations said that dairy calves in Norway should be fed 6 litres of milk (10 – 13% of bodyweight (BW)) per day divided on 3 meals to avoid overfilling the abomasum (Hansen et al., 2011). Recently the recommendation was adjusted to 8 litres per day (10 – 18% of BW) (Overrein et al., 2015). A thorough Canadian metastudy recommend feeding dairy calves the equivalent of 20% of BW per day (Khan et al., 2011). Such volumes are much more in line with the natural feeding behaviour of dairy calves. Calves allowed to suckle will generally do so 5-8 times (new-born) per day (Phillips, 1993). At 2-4 weeks of age calves can drink up to 8-10 litres of milk per day from an artificial teat (Appleby et al., 2001) or as much as 12-15 litres per day if they are allowed to suckle (Flower and Weary, 2001, Grøndahl, unpublished).

Advantages of feeding more milk

The results from Paper IV indicated that calves can be fed larger milk meals than 2-3 litres. These findings are of great practical importance as they show that farmers can adjust their feeding regime and feed their calves more milk by increasing the meal size. Hence, the calves can receive more milk without increasing the workload for farmers without automated milk feeders. Increased milk allowances are considered important to increase dairy calf

welfare (Khan et al., 2011). However, fear of milk entering the rumen has been a major reason for restrictive milk feeding. The Norwegian recommendations for milk feeding in dairy calves is 2 litres of milk 3-4 times per day (Overrein et al., 2015), whereas the legislation states that calves should be fed milk at least twice daily (Landbruks- og matdepartementet, 2004). This means that feeding dairy calves 2 meals per day is legal. Accordingly, results from a study conducted among Norwegian and Swedish producers of organic milk showed that feeding calves as low as 4 litres per day was not uncommon (Ellingsen et al. 2015). During the first few weeks of life, milk is the calf's primary source of nutrition. The intake of solid food is negligible and independent of the amount of milk (Flower and Weary, 2001). The young calves are hence unable to compensate for the low amounts of milk given to them by eating more grain and roughage. Until the calf is 3-4 weeks old, restrictive milk feeding can result in the calf being unable to meet the daily energy requirements (Nielsen et al., 2011), leading to chronic hunger (De Paula Vieira et al., 2008). Feeding more milk also has a number of advantages for calf health and welfare.

A major advantage of more milk is the increased growth rate. Research has shown that calves given milk equal to 10% of their BW have a daily growth rate (DGR) of 350-500 grams (Appleby et al., 2001; Flower and Weary, 2001; Jasper and Weary, 2002). Calves that have free access to milk from a teat were found to have a DGR of about 800 grams (Appleby et al., 2001; Jasper and Weary, 2002), while calves allowed to suckle freely showed a DGR of 1100-1200 grams (Flower and Weary, 2001; Grøndahl et al., 2007; Johnsen et al., 2015). It has also been shown that this unused growth potential during the first weeks of life cannot be compensated for later (Flower and Weary, 2001). Feeding higher amounts of milk has been found to improve feed conversion efficiencies (Khan et al., 2007) during the preweaning period and decrease mortality and disease susceptibility (Flower and Weary, 2001; Baldwin et al., 2004). More milk is also associated with reduced breeding age and higher milk yield later in life (Moallem et al., 2010).

Increased milk intake has been associated with higher levels of play behaviour (Krachun et al., 2010), an important indicator of good animal welfare. Further, more milk reduces the occurrence of tongue rolling, cross sucking and sucking on pen interior (de Passillé, 2001).

Large milk meals and pain behaviour

While radiography was included to investigate the physiological effects of high amounts of milk, the behavioural observations were carried out to detect signs of abdominal

pain or discomfort. Behaviour is frequently used as an indicator for animal welfare. For instance, play behaviour can indicate good animal welfare (Boissy et al., 2007), while pain behaviour can indicate poor animal welfare (Schwartzkopf-Genswein et al., 1997). If the results from Paper IV, either physiological or behavioural, indicated adverse effects of high amounts of milk, increasing the milk meal size beyond 2-3 litres would not be recommended.

On several occasions, the calves were seen licking their abdomen. This behaviour was executed as part of the grooming, and was not directed solely at the abdomen. Most calves would also lie down and get up again during the observation period. The behaviour was carried out with a low frequency and was not observed in association with the other pain behaviours. On most occasions, the calves would rise in response to external stimuli, e.g. people looking into the pen, or to drink water or eat hay. Taking these contextual factors into consideration, it is unlikely that the behaviours observed were actual signs of abdominal pain or discomfort. Whether abdominal pain occurred after the two-hour observation period is unknown. However, no irregularities were noticed by the technician during the midday or evening feeding following the morning test sessions, or in the days in-between testing.

Large milk meals and diarrhoea

An argument for restrictive milk feeding in calves is the belief that large amounts of milk can lead to more diarrhoea. In Paper IV, the calves were fed a milk:barium sulphate mix for radiographic purposes. As barium sulphate is antidiarrheal and could hide any laxative effects of increased amounts of milk, it is hard to draw conclusions from Paper IV. Also, the results from Paper IV were based on large milk meals fed once a day over a period of only three days. The long term effects of high milk rations were hence beyond the scope of Paper IV.

Studies investigating the link between high volumes of milk and diarrhoea report ambiguous results. While some confirm the connection (Diaz et al., 2001; Quigley et al., 2006), others fail to find it (Jasper and Weary, 2002; Uys et al., 2011; Bach et al., 2013). In the review article by Khan et al. (2011), it is suggested that a high incidence of diarrhoea is likely to be a problem more related to poor hygiene, management, housing conditions, ventilation and colostrum intake than to the amount of milk. It is also important to note that a calf drinking high amounts of milk will have more liquid faeces. This is not the same as diarrhoea caused by a pathological agent.

Integrated discussion

With respect to the three approaches to animal welfare (Figure 1), all aspects are covered by the four papers included in this thesis. Animal welfare as biological functioning (e.g. health, stress coping, growth, etc.) was covered in Papers I, III and IV. Animal welfare resulting from natural living and natural behaviour was covered in Papers I and III, while affective state was the centre of attention in Papers II and III.

Legislation defines the frames for how animals must be kept, and is therefore important to ensure good welfare. The animal's physical environment is vital for animal welfare and with generally stricter regulations, organic dairy farming has several prerequisites for better animal welfare compared to conventional dairy farming. However, as shown in Paper I, legislation alone is not enough to cause animal welfare to be perceived as better by veterinarians and agricultural advisors. Norway has a comprehensive legislation for conventional animal production, and the legal differences between the organic and conventional production systems are relatively small, perhaps too small to be picked up by the method applied. Nevertheless, the results from Paper I confirm international research which also fails to find significant differences between the two systems (Sundrum, 2001; Lund and Algers, 2003; Kijlstra and Eijck 2006). The missing difference between organic and conventional production systems does not mean that the organic regulations are without value. Rather, the results from Paper I indicate that the management at the level of the individual farmer is more important in determining calf welfare. This view was strengthened in Paper II, where stockperson handling style and the human-animal relationship were found to directly affect animal welfare. The link demonstrates the importance of good stockmanship and emphasises the responsibility each individual stockperson has in providing good welfare for the animals. The results also highlight the importance of shaping attitudes and motivating stockpeople, as this directly influences stockperson qualities and behaviour (Breuer et al., 2000; Coleman and Hemsworth, 2014).

Bhaskar's transformational model (Bhaskar, 2014) was included as a framework in this thesis for two reasons. Firstly, it shows that management is complex and works at both a structural and an individual level. Secondly, it shows that development is an integrated process between the two levels. For instance, if a management procedure is successfully carried out in a novel way, either by a farmer or as part of applied research, the procedure can be communicated to other farmers, who also adopt it. If sufficiently advantageous, the procedure may, over time, also be incorporated into the rules and regulations of the farming

structure, leading to development in the field of dairy calf management and welfare. This theory directly applies to Papers III and IV. By challenging the established ways of performing management routines, in this case cow-calf separation and milk feeding, the welfare of dairy calves can be improved and the field progresses.

The results in this thesis support the notion that management, especially at the individual level, has great impact on dairy calf welfare.

Conclusions and implications

The aim of this thesis was to contribute knowledge to the field of calf management and its impact on welfare. This has been achieved through various approaches and the main conclusions and implications are as follows:

Low calf mortality and adequate treatment of ill or injured animals were rated most favourably for calves in organic dairy production. Body condition and growth received the least favourable scores. The calf staying with its mother after birth was seen as the most important welfare advantage, while poor feed quality was seen as the most important welfare challenge. Veterinarians and agricultural advisors generally considered overall health and welfare to be equal for calves in organic and conventional dairy farming systems in Norway. Veterinarians and agricultural advisors diverged very little in their evaluations of calf health and welfare, hence not lending support to the idea that veterinarians are more sceptical about organic farming.

How we treat our calves has a direct effect on calf behaviour and mood and hence their level of welfare. Stockpeople who pet and talk to their animals, as well as handle them in a calm and patient manner, have calves that display higher frequencies of positive behaviour and mood as defined by descriptors like social, content and happy. On the other hand, stockpeople who treat their animals in an aggressive or dominating way, or those who are nervous and insecure, are more likely to have calves showing higher frequencies of negative behaviour and mood, as defined by descriptors like depressed, bored and boisterous. The positive and negative behaviour and mood are believed to have direct impact on the calves' level of welfare. It is therefore essential that this link between humans and animals is taught to people working with animals, being students, experienced stockpeople or foreign help.

Certain management routines like weaning and separation are bound to be stressful for the animals. However, through the generation of new knowledge we can make these transitions as painless as possible. Our results showed that separating cow and calf in a way that allows them to hear, see, and physically touch each other, may lead to reduced separation stress for the calves, hence increasing their level of welfare.

After separation from the cow, calves continue to feed on milk for some time. The way this milk is administered, as well as the amount, has great influence on calf welfare. It is commonly believed that calves should not receive more than 2-3 litres of milk per meal. Our results showed that the abomasum has great potential for expansion and that voluntary intake

of high volumes of milk through a teat with a small opening was insufficient to cause milk to enter the rumen. This suggests that farmers can increase the amount of milk they feed their calves through increased meal size, not necessarily through an additional meal. This new knowledge has been incorporated into the new dairy calf feeding recommendations. Feeding milk meal sizes of four litres is now recommended as an alternative to the previously recommended two litres.

.

Future perspectives

With regards to the papers included in this thesis, certain future studies can be stipulated:

In Paper I the perceptions and opinions of practicing veterinarians and agricultural advisors were used to pinpoint advantages and challenges of organic farming. This study could be used as a pilot study to identify target areas which could be pursued in a follow-up study. This would allow farm visits to be more focused in ways of content and geographical locations. For instance, with regards to feed, direct comparisons could be made between farms located in different parts of the country and more targeted, local measures could be made.

Paper II shows the potential to use Qualitative Behaviour Assessment to evaluate stockperson behaviour. Further validation is needed to see if Qualitative Behaviour Assessment efficiently and correctly can be applied to stockpeople to capture handling styles as suggested in the paper and to investigate whether Qualitative Behaviour Assessment is a valid tool for the assessment of the human-animal relationship. Further validation for use on animals is also required. Several practical and technical challenges need to be overcome, as discussed above.

In Paper III we found that allowing physical contact between cow and calf may alleviate post-separation stress for the calf. Further studies are needed on dairy cattle to find the optimal separation method and to investigate whether the stress of separation is outweighed by the benefits of staying together. Including physiological parameters like heart rate, cortisol and proportion of eye white as indicators of stress, would also provide valuable information in future research on cow-calf separation.

In Paper IV it was concluded that farmers can feed their calves higher amounts of warm whole milk through a teat bottle with a relatively small opening. A number of variables, however, were not investigated. For instance, it is currently unknown if feeding high volumes of milk replacer would yield the same results. It is also uncertain whether cold milk or increased drinking speed as from a teat with a bigger opening or drinking straight from an open bucket would result in milk in the rumen, due to leakage from the oesophageal groove. A second experiment examining these variables is therefore in the planning.

Reference list

Agresti, A., Franklin, C.A., 2012. Statistics: the art and science of learning from data. 2nd ed. Pearson Prentice Hall, Upper Saddle River, NJ.

Akssenowa, M.J., 1931. Zur Physiologie des Magens der Wiederkäuer. Arch. Tierernähr. Tier. 7, 295-304.

Altman, D.G., 1990. Practical statistics for medical research. Chapman and Hall, London ; New York.

Andreasen, S.N., Wemelsfelder, F., Sandøe, P., Forkman, B., 2013. The correlation of Qualitative Behavior Assessments with Welfare Quality® protocol outcomes in on-farm welfare assessment of dairy cattle. Appl. Anim. Behav. Sci. 143, 9-17.

Appleby, M.C., 2004. Science is not enough: how do we increase implementation? Anim. Welf. 13, Suppl. 1, 159-162.

Appleby, M.C., Weary, D.M., Chua, B., 2001. Performance and feeding behaviour of calves on ad libitum milk from artificial teats. Appl. Anim. Behav. Sci. 74, 191-201.

Aristotle, 1907. The history of animals Web edition. The University of Adelaide, Adelaide.

Assié, S., Bareille, N., Beaudeau, F., Seegers, H., 2009. Management- and housing-related risk factors of respiratory disorders in non-weaned French Charolais calves. Prev. Vet. Med. 91, 218-225.

Bach, A., Terré, M., Pinto, A., 2013. Performance and health responses of dairy calves offered different milk replacer allowances. J. Dairy Sci. 96, 7790-7797.

Baldwin, R.L., McLeod, K.R., Klotz, J.L., Heitmann, R.N., 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87, Suppl., E55-E65.

Bentham, J., 1907. Of the limits of the penal branch of jurisprudence, In: Schofield, P. (Ed.), An introduction to the principles of morals and legislation, Clarendon Press, Oxford.

Bertenshaw, C., Rowlinson, P., 2015. Exploring stock managers' perceptions of the human-animal relationship on dairy farms and an association with milk production. Anthrozoös 22, 59-69.

Bertenshaw, C.E., Rowlinson, P., 2008. Exploring heifers' perception of 'positive' treatment through their motivation to pursue a retreated human. Anim. Welf. 17, 313-319.

Bhaskar, R., 2014. The possibility of naturalism: a philosophical critique of the contemporary human sciences. 4th ed. Routledge, London.

Blood, D.C., Studdert, V.P., 1999. Saunders comprehensive veterinary dictionary. 2nd ed. Saunders, London.

Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., Aubert, A., 2007. Assessment of positive emotions in animals to improve their welfare. Physiol. Behav. 92, 375-397.

Boivin, X., Lensink, B.J., Veissier, I., 2000. The farmer and the animal: a double mirror, In: Human-animal relationship: stockmanship and housing in organic livestock systems. Proceedings of the Third NAHWOA Workshop, University of Reading, Clermont-Ferrand, pp. 5-13.

Bokkers, E.A.M., de Vries, M., Antonissen, I., de Boer, I.J.M., 2012. Inter- and intra-observer reliability of experienced and inexperienced observers for the qualitative behaviour assessment in dairy cattle. Anim. Welf. 21, 307-318.

Borne, J.J.G.C.v.d., Alferink, S.J.J., Gerrits, W.J.J., 2004. Identifying ruminal drinking by measurement of respiratory quotient and methane production in preruminant calves. J. Anim. Sci. 82, Suppl. 1, 365.

Bourne, D., 2013. Assessment of pain in ruminants. In: Wildpro - the electronic encyclopaedia and library for wildlife [Online]. Available: http://wildpro.twycrosszoo.org/S/00Man/PainRumOverviews/PainGeneral/02Assessment_PainRum.htm [Accessed 20.02. 2013].

Breuer, K., Hemsworth, P.H., Barnett, J.L., Matthews, L.R., Coleman, G.J., 2000. Behavioural response to humans and the productivity of commercial dairy cows. Appl. Anim. Behav. Sci. 66, 273-288.

Breuer, K., Hemsworth, P.H., Coleman, G.J., 2003. The effect of positive or negative handling on the behavioural and physiological responses of nonlactating heifers. Appl. Anim. Behav. Sci. 84, 3-22.

Broom, D.M., 1986. Indicators of poor welfare. Br. Vet. J. 142, 524-526.

Broom, D.M., 1991. Animal welfare: Concepts and measurements. J. Anim. Sci. 69, 4167-4175.

Broom, D.M., 1998. Welfare, stress, and the evolution of feelings. Stress and Behavior 27, 371-403.

Broom, D.M., Fraser, A.F., 2007. Domestic animal behaviour and welfare. 4th ed. CABI, Wallingford.

Brscic, M., Wemelsfelder, F., Tessitore, E., Gottardo, F., Cozzi, G., Van Reenen, C.G., 2009. Welfare assessment: correlations and integration between a Qualitative Behavioural Assessment and a clinical/ health protocol applied in veal calves farms. Ital. J. Anim. Sci. 8, Suppl. 2, 601-603.

Bush, L.J., Staley, T.E., 1980. Absorption of colostral immunoglobulins in newborn calves. J. Dairy Sci. 63, 672-680.

Byrne, B.M., 2010. Structural equation modeling with AMOS : Basic concepts, applications, and programming. 2nd ed. Taylor and Francis, Hoboken.

Bøe, K.E., Færevik, G., 2003. Grouping and social preferences in calves, heifers and cows. Appl. Anim. Behav. Sci. 80, 175-190.

Christiansen, S.B., Boesen, K.H., 2001. Organic farming and animal welfare - common goals or contradictory terms? In: Pasquali, M. (Ed.), 3rd Congress of the European Society for Agricultural and Food Ethics (EurSafe), Florence, Italy, pp. 177-180.

Coleman, G.J., Hemsworth, P.H., 2014. Training to improve stockperson beliefs and behaviour towards livestock enhances welfare and productivity. Rev. Sci. Tech. 33, 131-137.

D'Silva, J., 2009. Modern farming practices and animal welfare. In: Gunning, J., Holm, S., Kenway, I. (Eds.), Ethics, law and society, Vol. 4, Ashgate, Aldershot, pp. 7-18.

Davis, S.L., Cheeke, P.R., 1998. Do domestic animals have minds and the ability to think? A provisional sample of opinions on the question. J. Anim. Sci. 76, 2072-2079.

Dawkins, M.S., 2004. Using behaviour to assess animal welfare. Anim. Welf. 13, 3-7.

de Passillé, A.M., 2001. Sucking motivation and related problems in calves. Appl. Anim. Behav. Sci. 72, 175-187.

de Passillé, A.M., Rushen, J., 2005. Can we measure human-animal interactions in on-farm animal welfare assessment?: Some unresolved issues. Appl. Anim. Behav. Sci. 92, 193-209.

De Paula Vieira, A., Guesdon, V., de Passillé, A.M., von Keyserlingk, M.A.G., Weary, D.M., 2008. Behavioural indicators of hunger in dairy calves. Appl. Anim. Behav. Sci. 109, 180-189.

Dewi, S., Aune, T., Bunæs, J.A., Smith, A.J., Larsen, S., 2014a. The development of response surface pathway design to reduce animal numbers in toxicity studies. BMC Pharmacol. Toxicol. 15, 18.

Dewi, S., Kristiansen, V., Lindkær-Jensen, S., Larsen, S., 2014b. Between- and within-patient *n*-level response surface pathway design in dose-finding studies. Open Access Journal of Clin. Trials. 6, 63-74.

Diaz, M.C., Van Amburgh, M.E., Smith, J.M., Kelsey, J.M., Hutten, E.L., 2001. Composition of growth of holstein calves fed milk replacer from cirth to 105-kilogram body weight. J. Dairy Sci. 84, 830-842.

Dixon, W.J., Mood, A.M., 1948. A Method for Obtaining and Analyzing Sensitivity Data Journal of the American Statistical Association 43, 109-126.

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

Duve, L.R., Weary, D.M., Halekoh, U., Jensen, M.B., 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.

Eagly, A.H., Chaiken, S., 1993. The psychology of attitudes. Harcourt Brace Jovanovich, Fort Worth, Tex.

Ellingsen, K., Johnsen, J.F., Schjøll, A., Grøndahl, A.M., Mejdell, C.M., 2015. Kalvestell i norsk og svensk økomelkproduksjon. Resultater fra en spørreundersøkelse, Veterinærinstituttet, Oslo.Veterinærinstituttets rapportserie 16-2015.

Ellingsen, K., Mejdell, C.M., Hansen, B., Grøndahl, A.M., Henriksen, B.I.F., Vaarst, M., 2012. Veterinarians' and agricultural advisors' perception of calf health and welfare in organic dairy production in Norway. Org. Agric. 2, 67-77.

Ellingsen, K., Zanella, A.J., Bjerkås, E., Indrebø, A., 2010. The relationship between empathy, perception of pain and attitudes toward pets among Norwegian dog owners. Anthrozoös 23, 231-243.

Enríquez, D., Hötzel, M.J., Ungerfeld, R., 2011. Minimising the stress of weaning of beef calves: a review. Acta Vet. Scand. 53, 28.

Enríquez, D.H., Ungerfeld, R., Quintans, G., Guidoni, A.L., Hötzel, M.J., 2010. The effects of alternative weaning methods on behaviour in beef calves. Livest. Sci. 128, 20-27.

European Commission. 2008. Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control [Online]. Available: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2008.250.01.0001.01.ENG [Accessed 02.07. 2010].

Fleming, P.A., Paisley, C.L., Barnes, A.L., Wemelsfelder, F., 2013. Application of qualitative behavioural assessment to horses during an endurance ride. Appl. Anim. Behav. Sci. 144, 80-88.

Flower, F.C., Weary, D.M., 2001. Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth. Appl. Anim. Behav. Sci. 70, 275-284.

Flower, F.C., Weary, D.M., 2003. The effects of early separation on the dairy cow and calf. Anim. Welf. 12, 339-348.

Fraser, D., 2003. Assessing animal welfare at the farm and group level: the interplay of science and values. Anim. Welf. 12, 433-443.

Fraser, D., Weary, D.M., Pajor, E.A., Milligan, B.N., 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welf. 6, 187-205.

Gibbons, J.D., 1993. Nonparametric statistics: an introduction. Sage, Thousand Oaks, Calif.

Godden, S., 2008. Colostrum management for dairy calves. Vet. Clin. North Am. Food Anim. Pract. 24, 19-39.

Gorden, P.J., Plummer, P., 2010. Control, management, and prevention of bovine respiratory disease in dairy calves and cows. Vet. Clin. North Am. Food Anim. Prac. 26, 243-259.

Grandin, T., 2000. Livestock handling and transport. 2nd ed. CABI, Wallingford.

Grandin, T., 2010. The importance of measurement to improve the welfare of livestock, poultry and fish, In: Grandin, T. (Ed.), Improving animal welfare : a practical approach. 2nd ed. CABI, Wallingford.

Grøndahl, A.M., Skancke, E.M., Mejdell, C.M., Jansen, J.H., 2007. Growth rate, health and welfare in a dairy herd with natural suckling until 6-8 weeks of age: a case report. Acta Vet. Scand. 49, 16.

Gulliksen, S.M., 2010. Kalveoppstalling og kalvesjukdom i løsdrift. KuBygg-seminar, Stjørdal, Norway.

Gulliksen, S.M., Jor, E., Lie, K.I., Hamnes, I.S., Løken, T., Åkerstedt, J., Østerås, O., 2009a. Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. J. Dairy Sci. 92, 5057-5066.

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

Gutmann, A.K., Schwed, B., Tremetsberger, L., Winckler, C., 2015. Intra-day variation of Qualitative Behaviour Assessment outcomes in dairy cattle. Anim. Welf. 24, 319-326.

Haley, D.B., 2006. The behavioural response of cattle (*Bos taurus)* to artificial weaning in two stages. Department of Large Animal Clincal Sciences, University of Saskatchewan, Saskatoon. PhD thesis.

Haley, D.B., Stookey, J.M., 2005. Weaning cattle in two stages reduces the behavior changes typically associated with weaning stress. J. Dairy Sci. 88, 349-349.

Hanna, D., Sneddon, A., Beattie, V.E., 2009. The relationship between the stockperson's personality and attitudes and the productivity of dairy cows. Animal 3, 737-743.

Hansen, H.S., Havrevoll, Ø., Berg, J., Bævre, L., Nyhus, L.T., Gulliksen, S.M., 2011. Melkefôring av kalv : utredning basert på tilgjengelig litteratur og praktiske erfaringer. Utredning nr.127. Høgskolen i Nord-Trøndelag, Steinkjer.

Hektoen, L., 2004. Homeopathic treatment of farm animals. Studies of utilisation, effects and implications for animal health and welfare. Department of Production Clinical Sciences, Norwegian School of Veterinary Science, Oslo. PhD thesis.

Hemsworth, P.H., 2003. Human-animal interactions in livestock production. Appl. Anim. Behav. Sci. 81, 185-198.

Hemsworth, P.H., Barnett, J.L., Coleman, G.J., 1993. The human-animal relationship in agriculture and its consequences for the animal. Anim. Welf. 2, 33-51.

Hemsworth, P.H., Barnett, J.L., Hansen, C., 1981a. The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. Horm. Behav. 15, 396-403.

Hemsworth, P.H., Brand, A., Willems, P., 1981b. The behavioural response of sows to the presence of human beings and its relation to productivity. Livest. Prod. Sci. 8, 67-74.

Hemsworth, P.H., Coleman, G.J., 1998. Human–livestock interactions: the stockperson and the productivity of intensively farmed animals. CAB International, Wallingford.

Hemsworth, P.H., Coleman, G.J., Barnett, J.L., Borg, S., 2000. Relationships between human-animal interactions and productivity of commercial dairy cows. J. Anim. Sci. 78, 2821-2831.

Hu, L., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Struct. Equ. Modeling 6, 1-55.

IFOAM. 2009. Principles of organic agriculture [Online]. Available: http://www.ifoam.bio/sites/default/files/poa_english_web.pdf [Accessed 02.07. 2010].

IFOAM. 2012. The IFOAM norms for organic production and processing [Online]. Available: http://www.infonet-biovision.org/res/res/files/4228.ifoam_norms2012.pdf [Accessed 02.07. 2015].

IFOAM. 2014. IFOAM EU group - Norway [Online]. Available: http://www.ifoam-eu.org/en/norway [Accessed 02.07. 2015].

Irsik, M., Langemeier, M., Schroeder, T., Spire, M., Roder, J.D., 2006. Estimating the effects of animal health on the performance of feedlot cattle. Bovine Pract. 40, 65-74.

Jasper, J., Weary, D.M., 2002. Effects of ad libitum milk intake on dairy calves. J. Dairy Sci. 85, 3054-3058.

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

Jensen, P., 2002. The ethology of domestic animals: an introductory text. CABI Pub., Wallingford.

Johnsen, J.F., 2015. Suckling in dairy production. Welfare and management. Bonding and debonding, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences, Oslo. PhD thesis.

Johnsen, J.F., Beaver, A., Mejdell, C.M., Rushen, J., de Passillé, A.M., Weary, D.M., 2015. Providing supplementary milk to suckling dairy calves improves performance at separation and weaning. J. Dairy Sci. 98, 4800-4810.

Joinson, A., 1999. Social desirability, anonymity, and internet-based questionnaires. Behav. Res. Methods, Instrum. Comput. 31, 433-438.

Jolliffe, I.T., 2002. Principal component analysis, 2nd ed. Springer, New York.

Kachigan, S.K., 1986. Statistical analysis: An interdisciplinary introduction to univariate & multivariate methods. Radius Press, New York.

Khan, M.A., Lee, H.J., Lee, W.S., Kim, H.S., Kim, S.B., Ki, K.S., Ha, J.K., Lee, H.G., Choi, Y.J., 2007. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90, 876-885.

Khan, M.A., Weary, D.M., von Keyserlingk, M.A.G., 2011. Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. J. Dairy Sci. 94, 1071-1081.

Kijlstra, A., Eijck, I.A.J.M., 2006. Animal health in organic livestock production systems: a review. NJAS - Wageningen J. Life Sci. 54, 77-94.

Kiley, M., 1972. The vocalization of ungulates, their causation and function. Z. Tierpsychol. 31, 171-222.

Klein-Jöbstl, D., Iwersen, M., Drillich, M., 2014. Farm characteristics and calf management practices on dairy farms with and without diarrhea: A case-control study to investigate risk factors for calf diarrhea. J. Dairy Sci. 97, 5110-5119.

Krachun, C., Rushen, J., de Passillé, A.M., 2010. Play behaviour in dairy calves is reduced by weaning and by a low energy intake. Appl. Anim. Behav. Sci. 122, 71-76.

KRAV. 2015. 5.8 Mjölkuppfödda djur. [Online]. Available: http://www.krav.se/node/75505 [Accessed 02.07 2015].

Krohn, C.C., 2001. Effects of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioural aspects in high producing dairy cows - a review. Appl. Anim. Behav. Sci. 72, 271-280.

Landbruks- og matdepartementet. 2004. Forskrift om hold av storfe. FOR 2004-04-22-665. [Online]. Available: http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20040422-0665.html [Accessed 02.07 2015].

Landbruks- og matdepartementet. 2010. Lov om dyrevelferd. LOV-2009-06-19-97 [Online]. Available: https://lovdata.no/dokument/NL/lov/2009-06-19-97?q=Lov+om+dyrevelferd [Accessed 02.07 2015].

Landbruks- og matdepartementet. 2008. Om jordbruksoppgjøret 2008 endringer i statsbudsjettet for 2008 m.m. St.prp. nr. 69 (2007-2008). [Online]. Available: https://www.regjeringen.no/nb/dokumenter/stprp-nr-69-2007-2008-/id515511/?docId=STP200720080069000DDDEPIS&q=&navchap=1&ch=10. [Accessed 02.07 2015].

Landbruks- og matdepartementet. 2009. Økonomisk, agronomisk – økologisk! Handlingsplan for å nå målet om 15 pst. økologisk produksjon og forbruk i 2020. [Online]. Available: https://www.regjeringen.no/globalassets/upload/lmd/vedlegg/brosjyrer_veiledere_rapporter/handlingsplan_okologisk_200109.pdf [Accessed 02.07 2015].

Lateur-Rowet, H.J., Breukink, H.J., 1983. The failure of the oesophageal groove reflex, when fluids are given with an oesophageal feeder to newborn and young calves. Vet. Q. 5, 68-74.

Lensink, B.J., Boivin, X., Pradel, P., Le Neindre, P., Veissier, I., 2000a. Reducing veal calves' reactivity to people by providing additional human contact. J. Anim. Sci. 78, 1213-1218.

Lensink, B.J., Fernandez, X., Boivin, X., Pradel, P., Le Neindre, P., Veissier, I., 2000b. The impact of gentle contacts on ease of handling, welfare, and growth of calves and on quality of veal meat. J. Anim. Sci. 78, 1219-1226.

Lensink, B.J., Fernandez, X., Cozzi, G., Florand, L., Veissier, I., 2000c. The influence of farmers' behavior on calves' reactions to transport and quality of veal meat. J. Anim. Sci. 79, 642-652.

Lensink, B.J., Raussi, S., Boivin, X., Pyykkönen, M., Veissier, I., 2001a. Reactions of calves to handling depend on housing condition and previous experience with humans. Appl. Anim. Behav. Sci. 70, 187-199.

Lensink, B.J., Veissier, I., Florand, L., 2001b. The farmers' influence on calves' behaviour, health and production of a veal unit. Anim. Sci. 72, 105-116.

Lidfors, L.M., 1996. Behavioural effects of separating the dairy calf immediately or 4 days post-partum. Appl. Anim. Behav. Sci. 49, 269-283.

Loberg, J.M., Hernandez, C.E., Thierfelder, T., Jensen, M.B., Berg, C., Lidfors, L., 2007. Reaction of foster cows to prevention of suckling from and separation from four calves simultaneously or in two steps. J. Anim. Sci. 85, 1522-1529.

Loberg, J.M., Hernandez, C.E., Thierfelder, T., Jensen, M.B., Berg, C., Lidfors, L., 2008. Weaning and separation in two steps – A way to decrease stress in dairy calves suckled by foster cows. Appl. Anim. Behav. Sci. 111, 222-234.

Lund, V., 2002. Ethics and animal welfare in organic animal husbandry. An interdisciplinary approach, Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara. PhD thesis.

Lund, V., 2006. Natural living - a precondition for animal welfare in organic farming. Livest. Sci. 100, 71-83.

Lund, V., Algers, B., 2003. Research on animal health and welfare in organic farming - a literature review. Livest. Prod. Sci. 80, 55-68.

Lund, V., Anthony, R., Röcklinsberg, H., 2004a. The ethical contract as a tool in organic animal husbandry. Agric. Environ. Ethics 17, 23-49.

Lund, V., Hemlin, S., Lockeretz, W., 2002. Organic livestock production as viewed by Swedish farmers and organic initiators. Agriculture and Human Values 19, 255-268.

Lund, V., Hemlin, S., White, J., 2004b. Natural behavior, animal rights, or making money – A study of Swedish organic farmers' view of animal Issues. Agric. Environ. Ethics 17, 157-179.

Lundmark, F., Berg, C., Schmid, O., Behdadi, D., Röcklinsberg, H., 2014. Intentions and Values in Animal Welfare Legislation and Standards. Agric. Environ. Ethics 27, 991-1017.

Marchant-Forde, J.N., Marchant-Forde, R.M., Weary, D.M., 2002. Responses of dairy cows and calves to each other's vocalisations after early separation. Appl. Anim. Behav. Sci. 78, 19-28.

Marinelli, L., Adamelli, S., Normando, S., Bono, G., 2007. Quality of life of the pet dog: Influence of owner and dog's characteristics. Appl. Anim. Behav. Sci. 108, 143-156.

Martin, P., Bateson, P., 2007. Measuring behaviour: an introductory guide. 3rd ed. Cambridge University Press, Cambridge.

Mattilsynet. 2015. Veileder til forskrift om økologisk produksjon og merking av økologiske landbruksprodukter og næringsmidler, av 4. oktober 2005 nr. 1103. Veileder B. Utfyllende informasjon om økologisk landbruksproduksjon. [Online]. Available: http://www.mattilsynet.no/om_mattilsynet/gjeldende_regelverk/veiledere/veileder_b_utfyllende_informasjon_om_okologisk_landbruksproduksjon.2651/BINARY/Veileder%20B.%20Utfyllende%20informasjon%20om%20%C3%B8kologisk%20landbruksproduksjon [Accessed 02.07 2015].

Mavis, B.E., Brocato, J.J., 1998. Postal surveys versus electronic mail surveys: The tortoise and the hare revisited. Eval. Health Prof. 21, 395-408.

Minero, M., Tosi, M.V., Canali, E., Wemelsfelder, F., 2009. Quantitative and qualitative assessment of the response of foals to the presence of an unfamiliar human. Appl. Anim. Behav. Sci. 116, 74-81.

Moallem, U., Werner, D., Lehrer, H., Zachut, M., Livshitz, L., Yakoby, S., Shamay, A., 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93, 2639-2650.

Müllner, B., 2011. Comparison of free choice profiling and fixed terms for qualitative behaviour assessment in dairy cattle, Department of Sustainable Agricultural Systems, Univsersity of Natural Resources and Life Sciences, Vienna. Master thesis.

Munksgaard, L., de Passillé, A.M., Rushen, J., Herskin, M.S., Kristensen, A.M., 2001. Dairy cows' fear of people: social learning, milk yield and behaviour at milking. Appl. Anim. Behav. Sci. 73, 15-26.

Napolitano, F., De Rosa, G., Braghieri, A., Grasso, F., Bordi, A., Wemelsfelder, F., 2008. The qualitative assessment of responsiveness to environmental challenge in horses and ponies. Appl. Anim. Behav. Sci. 109, 342-354.

Napolitano, F., De Rosa, G., Caporale, G., Carlucci, A., Grasso, F., Monteleone, E., 2007. Bridging consumer perception and on-farm assessment of animal welfare. Anim. Welf. 16, 249-253.

Napolitano, F., De Rosa, G., Grasso, F., Wemelsfelder, F., 2012. Qualitative Behaviour Assessment of dairy buffaloes (*Bubalus bubalis*). Appl. Anim. Behav. Sci. 141, 91-100.

Nicol, A.M., 1977. Beef cattle weaning methods. N. Z. Agric. 134, 17-18.

Nielsen, H.M., Olesen, I., Navrud, S., Kolstad, K., Amer, P., 2011. How to consider the value of farm animals in breeding goals. A review of current status and future challenges. Agric. Environ. Ethics 24, 309-330.

Nodeland, R.H. 2013. Melkeroboter gir bønder økt trivsel [Online]. forskning.no. Available: http://forskning.no/husdyr/2013/08/melkeroboter-gir-bonder-okt-trivsel [Accessed 02.07. 2015].

OIE - World Organisation for Animal Health. 2010. Terrestrial Animal Health Code [Online]. Available: http://web.oie.int/eng/normes/mcode/en_chapitre_1.7.1.htm [Accessed 02.07 2015].

Overrein, H., Whist, A.C., Sølvberg, K.M., Nyhus, L.T., 2015. Godt kalveoppdrett – det er bedre å bygge kalver enn å reparere kyr, In: Overrein, H. (Ed.), Tine Rådgiving.

Padilla de la Torre, M., Briefer, E.F., Reader, T., McElligott, A.G., 2015. Acoustic analysis of cattle (Bos taurus) mother–offspring contact calls from a source–filter theory perspective. Appl. Anim. Behav. Sci. 163, 58-68.

Pajor, E.A., Rushen, J., de Passillé, A.M., 2000. Aversion learning techniques to evaluate dairy cattle handling practices. Appl. Anim. Behav. Sci. 69, 89-102.

Pallant, J., 2010. SPSS survival manual: a step by step guide to data analysis using SPSS. 4th ed. McGraw-Hill, Maidenhead.

Panamá Arias, J.L., Špinka, M., 2005. Associations of stock persons' personalities and attitudes. Czech J. Anim. Sci. 50, 226–234.

Panksepp, J. (Ed.), 1998. Affective neurosience: The foundations of human and animal emotions. Oxford University Press, New York.

Paul, R.K., Rosenberger, W.F., Flournoy, N., 2004. Quantile estimation following non-parametric phase I clinical trials with ordinal response. Stat. Med. 23, 2483-2495.

Phelps, N., 2007. The longest struggle. Animal advocacy from Phytagoras to PETA. Lantern Books, New York.

Phillips, C.J.C., 1993. Cattle behaviour. Farming Press, Ipswich.

Phythian, C., Michalopoulou, E., Duncan, J., Wemelsfelder, F., 2013. Inter-observer reliability of qualitative behavioural assessments of sheep. Appl. Anim. Behav. Sci. 144, 73-79.

Price, E.O., Harris, J. E., Borgwartdt, R. E., Sween, M. L., Connor, J. M., 2003. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behaviour and growth rate. Anim. Sci. 81, 116-121.

Price, P.C., 2012. Reliability and validity of measurement. In: Psychology research methods: Core concepts and skills, v. 1.0. Flat World Knowledge, Fresno.

Quigley, J.D., Wolfe, T.A., Elsasser, T.H., 2006. Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. J. Dairy Sci. 89, 207-216.

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

Raussi, S., 2003. Human-cattle interactions in group housing. Appl. Anim. Behav. Sci. 80, 245-262.

Reips, U.-D., Funke, F., 2008. Interval-level measurement with visual analogue scales in Internet-based research: VAS Generator. Behav. Res. Methods 40, 699-704.

Rigby, D., Cáceres, D., 2001. Organic farming and the sustainability of agricultural systems. Agric. Syst. 68, 21-40.

Rousing, T., Wemelsfelder, F., 2006. Qualitative assessment of social behaviour of dairy cows housed in loose housing systems. Appl. Anim. Behav. Sci. 101, 40-53.

Rushen, J., de Passillé, A.M.B., Munksgaard, L., 1999a. Fear of People by Cows and Effects on Milk Yield, Behavior, and Heart Rate at Milking. J. Dairy Sci. 82, 720-727.

Rushen, J., Munksgaard, L., Marnet, P.G., de Passillé, A.M., 2001. Human contact and the effects of acute stress on cows at milking. Appl. Anim. Behav. Sci. 73, 1-14.

Rushen, J., Taylor, A.A., de Passillé, A.M., 1999b. Domestic animals' fear of humans and its effect on their welfare. Appl. Anim. Behav. Sci. 65, 285-303.

Rutherford, K.M.D., Donald, R.D., Lawrence, A.B., Wemelsfelder, F., 2012. Qualitative behavioural assessment of emotionality in pigs. Appl. Anim. Behav. Sci. 139, 218-224.

Salanti, G., Ulm, K., 2005. A non-parametric framework for estimating threshold limit values. BMC Med. Res. Method. 5, 36.

Schumacker, R.E., Lomax, R.G., 2004. A beginner's guide to structural equation modeling. 2nd ed. Lawrence Erlbaum Associates, Inc., New Jersey.

Schwartzkopf-Genswein, K.S., Stookey, J.M., Welford, R., 1997. Behavior of cattle during hot-iron and freeze branding and the effects on subsequent handling ease. J. Anim. Sci. 75, 2064-2072.

Seabrook, M.F., 1972. A study to determine the influence of the herdsman's personality on milk yield. Agric. Labour Sci. 1, 45-59.

Seabrook, M.F., 1984. The psychological interaction between the stockman and his animals and its influence on performance of pigs and dairy cows. Vet. Rec. 115, 84-87.

Seabrook, M.F., 1986. The relationship between man and animals in managed systems, in: Cole, D.J.A., Brander, G.C. (Eds.), Bioindustrial ecosystems, Elsevier, Amersterdam, pp. 211-222. Ecosystems of the World 21.

Selwyn, N., Robson, K., 1998. Using e-mail as a research tool. University of Surrey. Social research update, 21.

Shannon, D.M., Johnson, T.E., Searcy, S., Lott, A., 2002. Using Electronic Surveys: Advice from Survey Professionals. Pract. Assess. Res. Eval. 8, 1.

Shi-ming, M., Sauerborn, J., 2006. Review of History and Recent Development of Organic Farming Worldwide. Agric. Sci. China 5, 169-178.

Simensen, E., 2004. Animal Care - The human factor in animal production. Nor. Vet. Tidsskr. 116, 5-10.

Sjaastad, Ø.V., Hove, K., Sand, O., 2010. Physiology of domestic animals. Second edition. 2nd. ed. Scandinavian Veterinary Press, Oslo.

Skarstad, G.Å., Terragni, L., Torjusen, H., 2007. Animal welfare according to Norwegian consumers and producers: Definitions and implications. Int. J. Sociol. Agric. Food 15, 74-90.

Smith, B.P., 2014. Large animal internal medicine. 5th ed. Mosby Elsevier, St. Louis, Mo.

Solano, J., Orihuela, A., Galina, C.S., Aguirre, V., 2007. A note on behavioral responses to brief cow-calf separation and reunion in cattle (*Bos indicus*). J. Vet. Behav. Clin. Appli. Res. 2, 10-14.

Srivastava, J., 2007. Vegetarianism and meat-eating in 8 religions [Online]. Web Edition. Available: http://www.hinduismtoday.com/modules/smartsection/item.php?itemid=1541 [Accessed 02.07. 2015].

Statens landbruksforvaltning. 2013. Produksjon og omsetning av økologiske landbruksvarer. Rapport for 2013. [Online]. Available: https://www.google.no/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CCUQFjAB&url=https%3A%2F%2Fwww.slf.dep.no%2Fno%2Fproduksjon-og-marked%2Fmelk%2Fmarked-og-pris%2F_attachment%2F36328%3F_ts%3D145403fef18%26download%3Dtrue&ei=PfmUVf_yGoKwsAHmxoCoAQ&usg=AFQjCNEP3IMOcX8FJA0Rg6aiDVsM8D6Luw&bvm=bv.96952980,d.bGg [Accessed 02.07. 2015].

Stěhulová, I., Lidfors, L., Špinka, M., 2008. Response of dairy cows and calves to early separation: Effect of calf age and visual and auditory contact after separation. Appl. Anim. Behav. Sci. 110, 144-165.

Stockman, C.A., Collins, T., Barnes, A.L., Miller, D., Wickham, S.L., Beatty, D.T., Blache, D., Wemelsfelder, F., Fleming, P.A., 2011. Qualitative behavioural assessment and quantitative physiological measurement of cattle naïve and habituated to road transport. Anim. Prod. Sci. 51, 240-249.

Stockman, C.A., McGilchrist, P., Collins, T., Barnes, A.L., Miller, D., Wickham, S.L., Greenwood, P.L., Cafe, L.M., Blache, D., Wemelsfelder, F., Fleming, P.A., 2012. Qualitative behavioural assessment of Angus steers during pre-slaughter handling and relationship with temperament and physiological responses. Appl. Anim. Behav. Sci. 142, 125-133.

Stookey, J.M., Schwartzkopf-Genswein, K. S., Waltz, C. S., Watts, J. M., 1997. Effects of remote and contact weaning on behaviour and weight gain of beef calves. J. Anim. Sci. 75, Suppl 1, 83.

Sundrum, A., 2001. Organic livestock farming: A critical review. Livest. Prod. Sci. 67, 207-215.

Sundrum, A., Padel, S., Arsenos, G., Kuzniar, A., Henriksen, B.I.F., Walkenhorst, M., Vaarst, M., 2006. Current and proposed EU legislation on organic livestock production, with a focus on animal health, welfare and food safety: a review, In: Rymer, C., Vaarst, M., Padel, S. (Eds.), The 5th SAFO Workshop, Odense. Fischer Taschenbuch Verlag - Forum Wissenschaft Hochschule, 75-90.

Svensson, C., Hultgren, J., Oltenacu, P.A., 2006. Morbidity in 3–7-month-old dairy calves in south-western Sweden, and risk factors for diarrhoea and respiratory disease. Prev. Vet. Med. 74, 162-179.

Temple, D., Dalmau, A., Ruiz de la Torre, J.L., Manteca, X., Velarde, A., 2011. Application of the Welfare Quality® protocol to assess growing pigs kept under intensive conditions in Spain. J. Vet. Behav. Clin. Appli. Res. 6, 138-149.

The Dairy Site. 2013. Just why do the danish lead the way? [Online]. Sheffield, England: 5m Publishing. Available: http://www.thedairysite.com/articles/3535/just-why-do-the-danish-lead-the-way/ [Accessed 02.07.2015].

Thomas, T.J., Weary, D.M., Appleby, M.C., 2001. Newborn and 5-week-old calves vocalize in response to milk deprivation. Appl. Anim. Behav. Sci. 74, 165-173.

Tine. 2015. Årsrapporter i Kukontrollen [Online]. Available: https://medlem.tine.no/minedata-kk/#/advisor/report/statistics/annualBreedsReport?selectedId=&grouping=Country&grouping=County&grouping=DairyGroup&grouping=DairyRegion&timeSpan=annualStatistics&type=breeds&timeSpanYear=2014&timeSpanMonth=&timeSpanUnitsInPast=0&resultBlocks=Breeds&breeds=1& [Accessed 12.02. 2015].

United States Environmental Protection Agency. 2010. Acute Oral Toxicity: Up-and-Down Procedure [Online]. Available: http://www.epa.gov/scipoly/sap/meetings/2001/december12/upanddown.pdf [Accessed 02.07.2015].

USDA Milk Production Report. 2014. 2013 U.S. Dairy Statistics [Online]. Available: http://www.progressivedairy.com/images/downloads/2014/06/02/1014pd_dairystats.pdf [Accessed 02.07.2015].

Uys, J.L., Lourens, D.C., Thompson, P.N., 2011. The effect of unrestricted milk feeding on the growth and health of Jersey calves. J. S. Afr. Vet. Assoc. 82, 47-52.

Vaarst, M., 1998. Omlægning til økologisk drift set fra konsulenters og dyrlægers synsvinkel. In: Kristensen, E.S., Thamsborg, S.M. (Eds.), Sundhed, velfærd og medicinanvendelse ved omlægning til økologisk drift. Research Centre Foulum Report 699 , 15-46.

Vaarst, M., Alban, L., Mogensen, L., Milan, S., Kristensen, E.S., 2001. Health and welfare in Danish dairy cattle in the transition to organic production: Problems, priorities and perspectives. Agric. Env. Ethics 14, 367-390.

Vaarst, M., Padel, S., Arsenos, G., Sundrum, A., Kuzniar, A., Walkenhorst, M., Grøva, L., Henriksen, B.I.F., 2006. Challenges for animal health and welfare in the implementation of the EU legislation on organic livestock production: Analysis of questionnaire survey among SAFO participants. In: Rymer, C., Vaarst, M., Padel, S. (Eds.). The 5th SAFO Workshop, Odense. Fischer Taschenbuch Verlag - Forum Wissenschaft Hochschule, 43-74.

Vaarst, M., Padel, S., Younie, D., Hovi, M., Sundrum, A., Rymer, C., 2008. Animal health challenges and veterinary aspects of organic livestock farming identified through a 3 year EU network project. Open Vet. Sci. J. 2, 111-116.

Veissier, I., Le Neindre, P., 1989. Weaning in calves: Its effects on social organization. Appl. Anim. Behav. Sci. 24, 43-54.

Ventura, B.A., von Keyserlingk, M.A.G., Schuppli, C.A., Weary, D.M., 2013. Views on contentious practices in dairy farming: The case of early cow-calf separation. J. Dairy Sci. 96, 6105-6116.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A.M., Visser, E.K., Jones, R.B., 2006. Assessing the human-animal relationship in farmed species: A critical review. Appl. Anim. Behav. Sci. 101, 185-242.

Waiblinger, S., Menke, C., Coleman, G., 2002. The relationship between attitudes, personal characteristics and behaviour of stockpeople and subsequent behaviour and production of dairy cows. Appl. Anim. Behav. Sci. 79, 195-219.

Walker, J., Dale, A., Waran, N., Clarke, N., Farnworth, M., Wemelsfelder, F., 2010. The assessment of emotional expression in dogs using a Free Choice Profiling methodology. Anim. Welfare 19, 75-84.

Weary, D.M., Chua, B., 2000. Effects of early separation on the dairy cow and calf: 1. Separation at 6 h, 1 day and 4 days after birth. Appl. Anim. Behav. Sci. 69, 177-188.

Weary, D.M., Jasper, J., Hotzel, M.J., 2008. Understanding weaning distress. Appl. Anim. Behav. Sci. 110, 24-41.

Wemelsfelder, F., 2007. How animals communicate quality of life: the qualitative assessment of behaviour. Anim. Welf. 16, 25-31.

Wemelsfelder, F., 1997. Investigating the animal's point of view. An enquiry into a subject-based method of measurement in the field of animal welfare, In: Dol, M., Kasanmoentalib, S., Lijmbach, S., Rivas, E., van den Bos, R. (Eds.), Animal consciousness and animal ethics: Perspectives from the Netherlands. Van Gorcum, Assen, 73-89.

Wemelsfelder, F., Hunter, A.E., Paul, E.S., Lawrence, A.B., 2012. Assessing pig body language: agreement and consistency between pig farmers, veterinarians, and animal activists. J. Anim. Sci. 90, 3652-3665.

Wemelsfelder, F., Hunter, E.A., Mendl, M.T., Lawrence, A.B., 2000. The spontaneous qualitative assessment of behavioural expressions in pigs: first explorations of a novel methodology for integrative animal welfare measurement. Appl. Anim. Behav. Sci. 67, 193-215.

Wemelsfelder, F., Hunter, T.E.A., Mendl, M.T., Lawrence, A.B., 2001. Assessing the 'whole animal': a free choice profiling approach. Anim. Behav. 62, 209-220.

Wemelsfelder, F., Knierim, U., Schulze Westerath, H., Lentfer, T., Staack, M., Sandilands, V., 2009a. Qualitative Behavour Assessment in: Forkman, B., Keeling, L. (Eds.), Assessment of animal welfare measures for layers and broilers, Cardiff University, United Kingdom, pp. 113-119.

Wemelsfelder, F., Lawrence, A.B., 2001. Qualitative assessment of animal behaviour as an on-farm welfare-monitoring tool. Acta Agric. Scand., A 51, 21-25.

Wemelsfelder, F., Millard, F., 2009. Qualitative behaviour assessment, in: Forkman, B., Keeling, L. (Eds.), Assessment of animal welfare measures for sows, piglets and fattening pigs, Cardiff University, United Kingdom, pp. 213-219.

Wemelsfelder, F., Millard, F., De Rosa, G., Napolitano, F., 2009b. Qualitative behaviour assessment, In: Forkman, B., Keeling, L. (Eds.), Assessment of animal welfare. Measures for dairy cattle, beef bulls and veal calves, Cardiff University, Cardiff, 215-224.

Wemelsfelder, F., Nevison, I., Lawrence, A.B., 2009c. The effect of perceived environmental background on qualitative assessments of pig behaviour. Anim. Behav. 78, 477-484.

Wickham, S.L., Collins, T., Barnes, A.L., Miller, D.W., Beatty, D.T., Stockman, C., Blache, D., Wemelsfelder, F., Fleming, P.A., 2012. Qualitative behavioral assessment of transport-naïve and transport-habituated sheep. J. Anim. Sci. 90, 4523-4535.

Wilson, S.D. 2002. Animals and Ethics [Online]. Available: http://www.iep.utm.edu/anim-eth/ [Accessed 22.06. 2015].

Zulkifli, I., 2013. Review of human-animal interactions and their impact on animal productivity and welfare. J. Ani. Sci. Biotechnol. 4, 25.

Østerås, O., 2009. Strukturforandringer i storfenæringen og konsekvenser for helsearbeidet. Husdyrforsøksmøtet 2009, Lillestrøm, Norway, 27-34.

Enclosed Papers I-IV

Veterinarians' and agricultural advisors' perception of calf health and welfare in organic dairy production in Norway

Kristian Ellingsen · C. M. Mejdell · B. Hansen · A. M. Grøndahl · B. I. F Henriksen · M. Vaarst

Abstract Veterinarians, as opposed to other professionals, have been accused of being unduly critical to animal welfare in organic farming. A nationwide questionnaire (QuestBack™) was distributed to 400 Norwegian production animal veterinarians and 400 agricultural dairy advisors to compare attitudes and opinions on calf health and welfare in organic dairy farming. The response rate was 52 % for veterinarians and 54 % for advisors. In direct comparison, both groups thought that the calves' overall health status and well-being did not differ in organic and conventional dairy farming systems. However, a significant number of both veterinarians and advisors considered the calves' confidence in people and space allowance to be better in organic dairy herds compared with conventional dairy herds, whereas feed quality was regarded to be poorer. When asked in more depth about organic dairy farming, the majority of respondents considered the routine of keeping calves with their mothers and the good care of the calves by stockpersons as important welfare advantages. Among all factors related to health, welfare, morbidity and mortality, low calf mortality and adequate treatment of disease and injury received the best scores. Body condition and growth, as well as the use of calf health recording cards, received the worst scores. The two professions differed in their views on the most important welfare challenges for calves in an organic environment: while both groups agreed on poor feed quality, veterinarians indicated concern for inadequate disease treatment while advisors pointed to poor hygiene. Results from the questionnaire indicated that the participants' familiarity with organic standards was relatively poor, especially among the less-experienced professionals. In conclusion, the view that veterinarians are more sceptical towards animal health and welfare in organic production compared with other professionals is not supported. The few differences found between the two groups may reflect different emphases due to their education and focus, e.g. treatment of disease versus advice on feeding, rather than different attitudes to organic farming. Future efforts should address the possible challenges and shortcomings in organic calf production found in the current study.

K. Ellingsen (✉) · C. M. Mejdell · A. M. Grøndahl
Norwegian Veterinary Institute,
PO Box 750, Sentrum,
0106 Oslo, Norway
e-mail: kristian.ellingsen@vetinst.no

B. Hansen
Bioforsk Nord Tjøtta,
PO Box 34,
8860 Tjøtta, Norway

B. I. F. Henriksen
Bioforsk Organic Food and Farming Tingvoll,
6630 Tingvoll, Norway

B. I. F. Henriksen · M. Vaarst
Department of Animal Sciences, AU Foulum,
Aarhus University,
PO Box 50, 8830 Tjele, Denmark

Springer

Introduction

Organic farming in Norway is steadily increasing. Between 1998 and 2009, approved organic farmland, including that in conversion, increased from 15,581 to 56,735 ha (Debio 2009). There has also been an unbroken increase in organic animal husbandry. In 2009, there were 7,622 organic dairy cows in Norway, 11.3 % higher than in 2008 (Debio 2009). Still, the proportion of organic production in Norway is small. In January 2010, only 3.2 % of the dairy cattle in Norway came from organic farms (Norwegian Agricultural Authority 2010). The most popular organic grocery category, dairy products, amounted to no more than 1.7 % of the total dairy product sales in 2009. There is still a long way to go before reaching the national goal of 15 % organic production by 2020.

The basic philosophy of organic farming is to develop robust, sustainable and environmentally friendly production systems while at the same time focusing on animal health and welfare (Ma and Joachim 2006;

Rigby and Cáceres 2001; IFOAM 2009). The Ministry of Agriculture and Food, in collaboration with Debio (the certification agency for organic production in Norway) and The Norwegian Food Safety Authority, has developed directives for this type of production system with respect to issues such as space allowance and access to outdoor areas, feed, medicine use and transportation. The directives are based on the EU Regulations on organic farming (Council Regulation (EC) No. 834/2007) of which Norway is bound by the EEA Agreement. The rules are meant to ensure animal welfare, as well as reduce the risk of medicine residue in food (Kijlstra and Eijck 2006). A list comparing rules for organic and conventional dairy farming in Norway is given in Table 1 (Norwegian Food Safety Authority 2009).

The level of animal welfare in organic herds has nevertheless been questioned.

Veterinarians were among the first to express concern (Vaarst et al. 2001) and are perceived as being sceptical to organic production systems (Sundrum et al. 2006). Recently, a prominent non-governmental organisation for animal welfare in Norway argued that the difference between organic and common animal welfare legislation has become too small, and consequently it no

Table 1 A comparison of rules for organic and conventional dairy cattle farming in Norway

Organic production	Conventional production
Calves older than 1 week cannot be kept in single boxes or pens. They must be allowed to see and physically touch other animals	Calves older than 8 weeks cannot be kept in single boxes or pens if there are other calves at approximately the same age in the herd. They must be allowed to see and physically touch other animals
All use of therapeutic substances/drugs must be registered in the Norwegian Cattle Health Recording System	Same as for organic
All animals must have access to a comfortable, clean, dry lying/resting area of sufficient size	Same as for organic, but bulls >6 months can be kept on slatted floors
Calves must be allowed to suckle for at least 3 days postpartum	Calves can be separated from the dam immediately after calving
For ruminants, at least 50 % of the feed must be produced on own farm or in cooperation with other organic farms	No equivalent rule for conventional production
The use of chemical or synthetically derived drugs must be limited. Natural therapeutic agents and methods are encouraged	No equivalent rule for conventional production
The withholding period for prescription drugs in organic farming is twice that in conventional systems	
Minimum half of the pen area must be solid floor	Cows and heifers (2 months before calving) and calves (0–6 months old) must have access to soft and solid lying/resting area. In new buildings, all female animals must have a solid floor on resting area

longer urges the public to choose organic animal products (http://www.dyrevern.no/artikler/nyheter_om_mat/fremtiden_for_oko-mat_i_norge). On the other hand, veterinarians have been criticised for having little knowledge about organic standards and welfare philosophy (Vaarst 1998), and a survey among European partners in a network project concerning animal health and food safety in organic farming clearly showed that veterinarians generally lacked adequate education in organic animal husbandry (Vaarst et al. 2006, 2008).

In light of the above, we wanted to explore both agricultural dairy advisors' and practising veterinarians' impressions of calf health and welfare in organic dairy herds, how they perceive their own level of familiarity with the regulations concerning organic farming and to find out whether the two groups of professionals differ in their views of the benefits and challenges with respect to organic dairy calves.

Another aim was to obtain information on calf rearing in organic herds, including critical points, as part of our work to develop a checklist, or protocol, for on-farm assessment of calf health and welfare. This would constitute an important tool in welfare planning and improvement at farm level (Vaarst et al. 2011; Ivemeyer et al. 2012). Veterinarians and agricultural advisors are in close contact with a considerable number of farms and different farming systems, and therefore constitute a valuable knowledge resource. Because of their experience, they are also in a strong position to evaluate the potential welfare advantages and challenges of different production methods.

Materials and methods

Data collection

In 2008, a nationwide Internet-based questionnaire (QuestBack™) was sent out to all 400 veterinarians in Norway who were members of the Norwegian Association of Large Animal Veterinary Practitioners and registered with an e-mail address. This group constitutes approximately 90 % of all veterinarians in large animal practice in the country. The same questionnaire was administered to a matching group of 400 agricultural advisors. The agricultural advisors were all employed by Tine, Norway's dominant producer, distributor and exporter of dairy products.

The questionnaire consisted of 50 questions and was divided into five parts. The issues and suggested alternative answers were selected to cover important aspects of calf health and welfare. Input was also obtained from experts during two workshops on calf health and welfare. A draft questionnaire was tested on a panel of three veterinarians and one agricultural advisor and the questionnaire was thereafter amended to increase clarity and to avoid ambiguity.

In part one of the questionnaire, respondents were asked to indicate on a scale from 1 ("very poor") to 6 ("very well") the level of their own knowledge of the organic regulations concerning dairy cattle husbandry and for medicine use. In part two, respondents were asked to rate the status of calves in organic herds for physical health, wellbeing, confidence in people, feed quality, feeding routines, hygiene and space allowance on a scale from 1 ("much worse") to 5 ("much better") compared with conventional dairy farms. In part three, participants were asked to express their general impression of cleanliness, skin and coat condition, digestive and respiratory problems, inflammation of the navel and joints, deficiency diseases, body condition and growth, mortality, quality of treatment of ill and injured animals and maintenance of calf health card recordings at organic dairy farms on a scale from 1 ("very poor") to 6 ("very good"). In part four, respondents were asked about the implementation of certain management routines with the alternatives "applies to all calves", "applies to most calves", "applies to some calves" and "applies to no calves". The questions covered the calves' opportunity to express natural behaviour, whether or not calves were separated from their mothers within 24 h postpartum and whether calves had access to outdoor areas outside the grazing season. Finally, in part five, participants were asked to select, from a list of statements (see Figs. 2 and 3), what they considered the most important welfare advantage and the most important welfare challenge for calves in organic housing systems.

Statistical analysis

Respondents with no practical experience from organic dairy farms during the last 5 years were excluded from the statistical analyses. A Wilcoxon rank-sum test was performed to test for potential differences based on profession (veterinarians vs. advisors), amount of experience with organic farms (1–3 vs. >3 farms) and years

since graduation (<3 vs. >20 years) with respect to how well they knew the regulations concerning organic dairy cattle husbandry and medicine use. To better characterise the respondents, variance was also examined. Potential differences between organic and conventional farming systems were tested for by Wilcoxon signed-rank test (reference category 3—"equal to"). The Wilcoxon rank-sum test was also used to test for effects of profession, exposure to organic farms and years since graduation on how respondents ranked the factors related to calf health, welfare, morbidity and mortality. To test whether the veterinarians and advisors had rated the welfare advantages or challenges differently, a Pearson's chi-square test was applied to all statements with more than ten responses.

Results

Characteristics of the respondents

A total of 207 (52 %) veterinarians and 215 (54 %) advisors responded to the questionnaire. Of these, 114 veterinarians (67 % male and 33 % female) and 112 advisors (64 % male and 36 % female) met the inclusion criteria "experience from organic farms in the last 5 years".

The veterinarians and advisors were generally very experienced. Of the respondents included in the study, 163 (72 %) had at least 10 years of experience with dairy farms and of these and 97 (43 %) had more than 20 years of experience. Seventy (61 %) of the veterinarians had experience from one to three organic farms, 39 (34 %) had experience from four to ten organic farms and 5 (4 %) had experience from more than ten organic farms. The corresponding figures for the advisors were 72 (64 %), 31 (28 %) and 9 (8 %), respectively. Respondents were divided into four groups based on their experience with organic dairy farms (see Table 2).

Familiarity with the regulations concerning organic dairy cattle husbandry

As shown in Fig. 1, experienced veterinarians regarded their familiarity with the legislation related to organic dairy cattle husbandry and on medicine use as better compared with those with less experience. The same pattern was found for the agricultural advisors. Advisors, regardless of experience, found themselves to be more familiar with the regulations concerning organic dairy cattle husbandry than did the veterinarians ($p<0.001$), but no differences were found concerning medicine use. The variance for veterinarians with respect to familiarity with regulations concerning organic dairy cattle husbandry and for medicine use was 1.95 and 2.14, respectively. For advisors, the respective variance for organic dairy cattle husbandry and medicine use was 1.17 and 1.64. No effect of years since graduation was found.

Comparison of organic and conventional dairy farming systems

As shown in Table 3, a significant number of respondents, both veterinarians and advisors, reported that they found feed quality (i.e. roughage quality) to be worse, but space allowance and calves' confidence in people to be better on organic farms compared with conventional farms. For the other factors, no significant differences were found.

Professionals with more than 20 years since graduation rated the physical health ($p<0.05$) and confidence in people ($p<0.01$) of calves on organic farms significantly better than professionals with less than 3 years since graduation.

Calf health, welfare, morbidity and mortality

As shown in Table 4, the factors that were judged to be best among organic dairy calves were low mortality and

Table 2 Grouping of the respondents based on profession and experience from farms with organic milk production

Group	Number
Veterinarians with experience from 1 to 3 farms (less experienced)	70
Veterinarians with experience from >3 farms (experienced)	44
Advisors with experience from 1 to 3 farms (less experienced)	72
Advisors with experience from >3 farms (experienced)	40

Fig. 1 Veterinarians' and advisors' evaluations on a scale from one ("very poor") to six ("very well") of their own familiarity with the regulations concerning organic dairy cattle husbandry and medicine use. The figure compares Low Experience veterinarians (N=70), Low Experience advisors (N=72), experienced veterinarians (N=44) and experienced advisors (N=39). [a]P<0.001
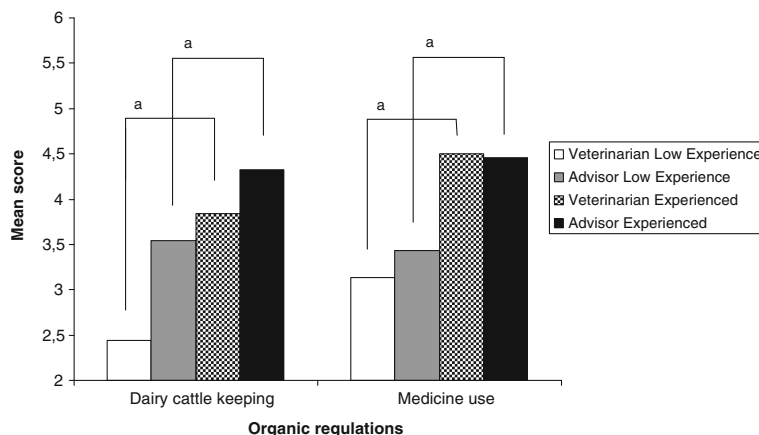
[a] (P < 0.001)

adequate treatment when animals were ill or injured. The factors thought to be worst were body condition and growth as well as routines for calf health card records.

A high degree of consensus was found to exist between veterinarians and advisors, and only two parameters were judged differently. The first issue, skin and coat condition, was considered significantly better by the advisors than by the veterinarians. The second issue, treatment of ill and injured animals, was also judged more favourably by the advisors. No effect from level of experience with organic farms was discovered.

Professionals with more than 20 years since graduation rated their general impression of cleanliness ($p<0.05$), skin and coat condition ($p<0.05$), digestive and respiratory problems ($p<0.05$) and inflammation of the navel and joints ($p<0.05$) as significantly better than professionals with less than 3 years since graduation.

Housing and behaviour

The results regarding housing and behaviour are given in Table 5.

Welfare advantages

Veterinarians and advisors did not evaluate the welfare advantages of calves on organic farms differently (χ-squared=4.3; n.s.). As shown in Fig. 2, the largest proportion of the respondents considered the routine of keeping calves with their mothers after parturition as the most important factor. The second largest proportion of respondents scored stockpersons caring well for the calves as the most important factor.

Welfare challenges

Veterinarians and advisors had significantly different perceptions regarding the most important welfare challenge in organic farming (χ-squared=11.9; $p<0.02$). As shown in Fig. 3, poor feed quality was seen as the most important welfare challenge by the largest proportion of the respondents, both veterinarians and advisors. The second factor most commonly chosen by veterinarians and advisors, respectively, was inadequate disease treatment and poor hygiene.

Table 3 Veterinarians' and advisors' opinions of feed quality, space allowance and calves' confidence in people in organic farming systems compared with conventional farming systems, on a scale from 1 to 5, 1 being "much worse", 3 being "equal to" and 5 being "much better" than in conventional farming systems

Factor	Veterinarians			Advisors		
	n	Mean	P[a]	n	Mean	P[a]
Feed quality	102	2.76	0.001	102	2.52	<0.001
Space	104	3.37	<0.001	103	3.31	<0.001
Calves' confidence in people	100	3.22	0.002	102	3.14	0.022

[a] Reference category 3—"equal to"

Table 4 Veterinarians' and advisors' opinions of parameters related to health, morbidity and mortality in organic farming on a scale from 1 ("very poor") to 6 ("very good")

	Veterinarians		Advisors		P
	n	Mean (SD)	n	Mean (SD)	
Mortality	87	4.20 (1.25)	81	4.32 (1.29)	n.s.
Treatment when ill or injured	97	3.73 (1.45)	89	4.29 (1.41)	0.008
Skin and coat condition	100	3.46 (1.00)	89	3.81 (1.04)	0.017
Body condition and growth	97	3.18 (1.02)	99	3.26 (1.14)	n.s.
Calf health card recordings	83	3.16 (1.40)	76	3.12 (1.37)	n.s.

Discussion

The respondents

A response rate of 54 % for advisors and 52 % for veterinarians is sufficiently high to generalise the results for Norwegian conditions. When respondents who had no experience from organic dairy farms during the last 5 years were excluded, the useable response rate of advisors and veterinarians was 28 and 29 %, respectively. This reflects the limited number of organic dairy farms in Norway.

It is important to emphasise that the results in this study shed light on veterinarians' and advisors' *perception* of the current situation, and not the actual situation. The opinions and assessments given by the respondents are influenced by both knowledge and attitudes. An example of this is that respondents who graduated more than 20 years ago rated the calves' health condition and confidence in people as significantly better than respondents who graduated less than 3 years ago. This may be due to the fact that the former group was educated at a time where animal welfare was mainly judged on the basis of physical health rather than a combination of physical and mental well-being. During the last few years, animal welfare science has gained significant grounds in veterinary education (Siegford et al. 2005) and may be the likely cause of the cohort effect seen in the study. Longer practical experience from a greater range of farms may be another reason for the differing views.

### Familiarity with the regulations concerning organic production

An important basis for attitude is knowledge. Even though the experienced groups regarded themselves as having a better familiarity with the regulations concerning organic dairy farming than the Low Experience groups, the general level of knowledge should be improved. The number of organic dairy farms on the national level is still quite small and the organic production system is unfamiliar to most veterinarians and advisors. Still, a major focus of organic farming is to secure good animal health and welfare. This is ensured by compliance with the rules and regulations concerning organic farming and by educating farmers. An aim for those working with farmers on organic dairy farms should be to increase their level of

Table 5 Veterinarians' and advisors' opinions on the proportion of calves in organic systems which are given the opportunity to express natural behaviour, be together with the mother for more than 1 day and given outdoor access outside the grazing season

Applies to	Opportunity to express natural behaviour		Separation from mother after <24 h		Outdoor access beyond grazing season	
	Veterinarians % (n=96)	Advisors % (n=51)	Veterinarians % (n=81)	Advisors % (n=43)	Veterinarians % (n=84)	Advisors % (n=45)
All calves	27.1	9.8	4.9	4.7	1.2	2.2
Most calves	47.9	66.7	29.6	23.3	7.1	11.1
Some calves	24.0	23.5	34.6	41.9	48.8	51.1
No calves	1.0	0.0	30.9	30.2	42.9	35.6

Responses are given on a scale from 1 ("applies to all calves") to 4 ("applies to no calves"). Values are given as percentages of the total number of responses

Fig. 2 Veterinarians' and advisors' opinions on the most important welfare advantage for calves in organic farming systems from a list of nine statements
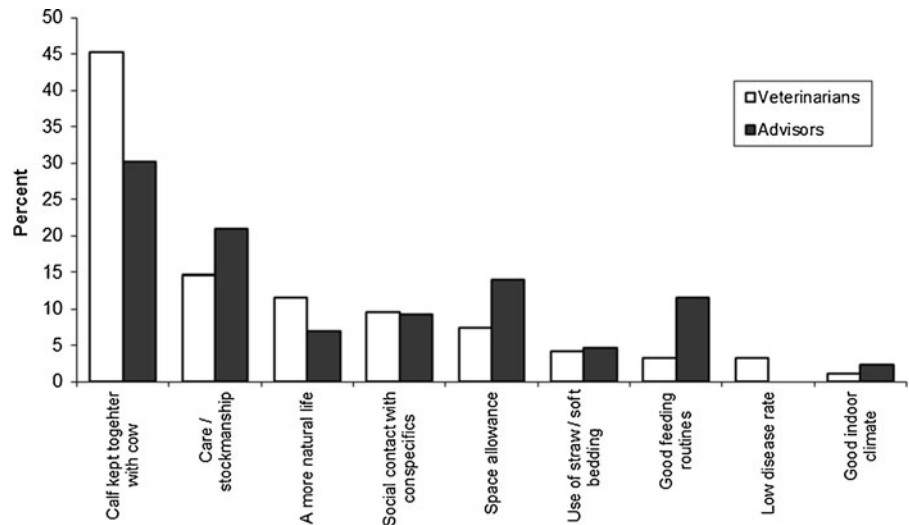
knowledge of regulations concerning organic production. Without knowing the fundamentals of organic production and the legislation concerning it, professionals will find it difficult to provide relevant advice to their clients. This has also been addressed as an issue in other publications (Sundrum et al. 2006).

Calf health, welfare, morbidity and mortality

Veterinarians and advisors had a relatively positive impression of the health and welfare of organic dairy calves as reflected by their opinions on calf mortality. In Norway in 2006, the mortality of calves from birth to 180 days across all systems was 2.1 %. In addition, 0.6 % were aborted and another 2.8 % were stillborn (Østerås et al. 2007). In economic terms, calf mortality costs Norwegian dairy farmers over 19 million Euro

annually (Østerås et al. 2007). Nevertheless, compared with other countries including Denmark (Ersboll et al. 2003), Ireland (Mee et al. 2008) and the USA (The Cattle Site 2009), the level of calf mortality is low in Norway. Low calf mortality was also the factor considered most favourable in organic dairy farming, by both the veterinarians and the agricultural advisors.

Calf mortality may be affected by farm management. Increasing herd sizes and the use of technological aids, such as automatic feeding systems, may lead to a decrease in the amount of time farmers and stockpersons spend in close contact with the animals. This in turn will lead to reduced observation of the individual animal, which again may influence mortality. A Norwegian study published in 2007 concluded that calf mortality in Norwegian dairy herds could be reduced by means of increased surveillance and improved

Fig. 3 Veterinarians' and advisors' opinions on the one most important welfare challenge for calves in organic farming systems from a list of eight statements
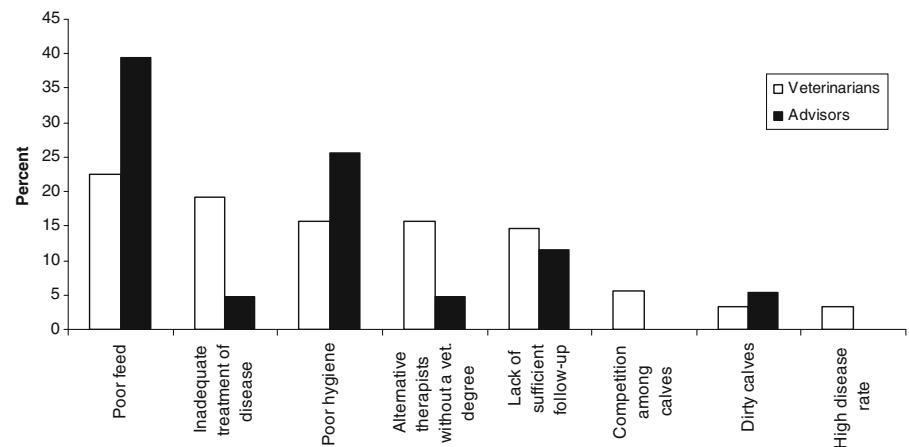
management during calving, as well as in the suckling period (Gulliksen et al. 2009a).

Keeping health records is a good way of monitoring animal health and welfare. The low scores for health registration cards found in the survey may indicate that diseases and treatments of calves are not recorded in a satisfactory manner in organic herds. While the Norwegian Cattle Health Recording System for dairy cows is well established and generally considered to be very reliable, a 40 % underreporting of calf disease and treatment has been shown to exist (Gulliksen et al. 2009b). Recording calf events is important for a number of reasons. Recordings provide the individual farmer with information about his own herd, thereby laying the foundation for control, planning and quality assurance. It also forms a basis for comparison with other producers. Recordings provide vets and advisors with valuable information about problem areas in a herd, and are also essential for optimising management through preventive health measures, research issues, and breeding (Gulliksen et al. 2009b). Health recording for calves is therefore key to calf health and welfare in the long term and an area with great potential for improvement, both in conventional and organic dairy farming.

Housing and behaviour

The calves' opportunity to express natural behaviour was seen as available to most organic dairy calves by both the veterinarians and the advisors. Natural behaviour holds a key position in organic farming and is essential to animal welfare (Lund 2006; Lund and Algers 2003; IFOAM 2009). Confirmation that this is frequently allowed is therefore of great importance. However, access to outdoor areas outside the grazing season was reported to be available to only a limited number of calves. This issue has not been included in governmental regulations, but the regulations concerning organic production state that all animals *should* have outdoor access outside the grazing season (Norwegian Food Safety Authority 2009). Lund (2006) discussed the view that the persons taking care of the animals were under an ethical obligation to allow them to live as naturally as possible under farming conditions. Animals with outdoor access are considered to lead richer, more natural lives with better opportunities to display a wider repertoire of natural behaviour. More organic farmers should therefore aim to follow this recommendation.

The participants responded that a significant number of calves were taken from their mothers within 24 h postpartum. Only about 30 % of the veterinarians and advisors had experience from farms that let all calves stay with their mothers for more than 24 h. The questionnaire, however, asked only about separation and so did not provide information on whether the 3-day suckling rule was complied with by bringing the calf to its mother several times a day. In any case, the results indicate frequent rule breaking (Table 5). This impression was confirmed by another survey conducted among Norwegian organic dairy producers, where 10 % of the farmers admitted that they did not comply with the 3-day suckling rule, but separated cow and calf immediately after birth (Henriksen, personal communication). As organic farming, to some extent, tries to mirror nature, cow and calf should be allowed to stay together for as long as possible. A white paper from the Norwegian Government (Ministry of Agriculture 2002–2003) regarding animal husbandry and animal welfare, also states that rearing systems allowing for more cow-calf contact after calving are desirable. The practice of permitting the calf and mother to be together was judged to be the most important welfare advantage for calves on organic farms by both veterinarians and advisors. On the other hand, separation after a social bond has been formed will inevitably lead to separation stress. However, disputing the optimal time for cow–calf separation is beyond the scope of this article. The low number of participants who replied to these questions (Table 5) might also indicate that many respondents felt unsure about the housing- and behaviour-related issues.

Welfare advantages

In this study, the largest proportion of respondents considered that the calf being allowed to stay with its mother after birth and the stockperson caring well for the animals were the most important welfare factors in organic farming. This harmonises well with the finding that the calves' confidence in people was one of the two factors found to be better in organic compared with conventional dairy farming. Animals handled in a positive way are safer and easier to handle, while a lack of habituation to people and negative handling with shouting and hitting, leads to lower levels of animal welfare, higher levels of fear and acute and chronic stress (Hemsworth 2003; Hemsworth et al.

2000; Lensink et al. 2000a, b; Simensen 2004). Fear of humans may also have direct effects on production, and studies have shown that a negative human–animal relationship can have consequences such as reduced milk yield and increased residual milk (Hemsworth et al. 2000; Rushen et al. 1999). Sundrum (2001) concluded in his review that the comparative studies examining health status in organic and conventional dairy farming found no fundamental differences between the two production methods. Organic farming has more stringent minimum standards providing preconditions for good animal welfare; however, animal health depended on management rather than the production system (Sundrum 2001).

Welfare challenges

Both the veterinarians and the advisors were concerned about poor quality feed. Poor quality feed as the number one welfare challenge is also in accordance with feed quality being the only factor considered worse in organic production compared with conventional systems by both veterinarians and advisors. That body condition and growth received poor ratings among the health parameters also supports this. Different factors may explain the poor feed quality. Firstly, the rule that at least 50 % of the feed has to be produced on the farm or in cooperation with other organic farms (Table 1) can constitute a problem as organic farms are few and widespread geographically in Norway. Buying supplementary organic feed can also be both difficult and expensive. Secondly, Norwegian dairy farms are often located in mountainous areas where it is difficult to grow anything other than grass. Finally, a lot of farmers convert from conventional to organic farming without increasing farmland and pasture size or reducing stock numbers. To grow nutritious feed is easier in countries with a more favourable climate such as Denmark and the Netherlands. Actually, a literature review by Worthington (1998) concluded that animals given organic feed demonstrated better growth and reproduction than animals receiving conventional feed.

With the exception of vaccines and anthelmintics, and treatment required by the government, routine or prophylactic use of chemical or synthetic drugs in organic farming is prohibited (Table 1). Sick animals may be treated with chemical or synthetic drugs for animal welfare reasons, otherwise other methods are preferred. Doubled withholding periods make medical disease treatment more expensive and thus less attractive, especially for lactating cows. Because of this, organic farming has been criticised, often by veterinarians, who claim that the animals do not get proper disease treatment (Lund and Algers 2003). Organic farmers have also been criticised because their animals have been malnourished and had a higher occurrence of parasites due to the restrictive use of anthelmintic drugs. In a Danish survey among practicing veterinarians and advisors, Vaarst (1998) found that the veterinarians who expressed this type of concern (e.g. that organic animals were not properly treated in medical terms) were unable to give actual examples based on their own experience during in-depth interviews. Among the advisors, poor treatment of disease was not a major concern. This may reflect that, in addition to veterinarians being actively confronted with disease-related issues, the work of veterinarians is more focussed on disease than that of advisors.

Differences between veterinarians and advisors

A high degree of consensus was found between how veterinarians and advisors perceive calf health and welfare in organic dairy farming. In general, the idea that veterinarians are more negative towards organic farming than agricultural professionals is not supported. However, a few differences were discovered. Advisors considered themselves to be more familiar with the regulations concerning organic dairy cattle husbandry than did veterinarians. This may be due to the fact that a veterinarian's primary function is to treat sick animals, while an agricultural advisor's primary function is to guide and strategically help farmers, a job requiring greater overall knowledge of legislation related to organic production. However, the trend was not found with respect to the rules for medicine use, an area in which the veterinarians need to stay updated. Nevertheless, in order to properly advise organic farmers, veterinarians would also benefit from having more insight into the specific regulations for organic production. This could be addressed in the veterinary curriculum and/or by means of post-qualifying courses.

Inadequate treatment of calf disease was a major concern among the veterinarians and was the factor second most commonly selected as the most important challenge in organic farming systems. Conversely, among the advisors, this was rated as the fifth most important factor. Inadequate treatment of sick and

injured animals was also rated as a challenge by more veterinarians than advisors. Not surprisingly, disease and disease treatment is of greater concern among veterinarians. In addition, scepticism of alternative animal therapists without a veterinary degree was more prominent among the veterinarians, likely reflecting the veterinarians' education in evidence-based traditional Western medicine.

As mentioned above, the current data are based on the *perceptions* of veterinarians and agricultural advisors. Because of their close contact with producers, it can be argued that their opinions are accurate and valuable. In future, efforts should be aimed at evaluating and improving the actual welfare in organic calf production, with focus on the challenges and shortcomings indicated by the current study.

References

Debio (2009) Statistikk 2009. Available at http://www.debio.no/_upl/statistikkhefte_2009.pdf. Accessed 23 July 2012

Ersboll A, Rugbjerg H, Stryhn H (2003) Increased mortality among calves in Danish cattle herds during bovine virus diarrhoea infection. Acta Vet Scand 44(Suppl 1):49

Gulliksen SM, Lie KI, Løken T, Østerås O (2009a) Calf mortality in Norwegian dairy herds. J Dairy Sci 92(6):2782–2795

Gulliksen SM, Lie KI, Østerås O (2009b) Calf health monitoring in Norwegian dairy herds. J Dairy Sci 92(4):1660–1669

Hemsworth PH (2003) Human–animal interactions in livestock production. Appl Anim Behav Sci 81(3):185–198

Hemsworth PH, Coleman GJ, Barnett JL, Borg S (2000) Relationships between human–animal interactions and productivity of commercial dairy cows. J Anim Sci 78(11):2821–2831

IFOAM (2009) Principles of organic agriculture. Available at http://www.ifoam.org/organic_facts/principles/pdfs/IFOAM_FS_Principles_forWebsite.pdf. Accessed 23 July 2012

Ivemeyer S, Smolders G, Brinkmann J, Gratzer E, Hansen B, Henriksen BIF, Huber J, Leeb C, March S, Mejdell C, Nicholas P, Roderick S, Stöger E, Vaarst M, Whistance LK, Rogerson I, Winckler C, Walkenhorst M (2012) Impact of animal health and welfare planning on medicine use, herd health and production in European organic dairy farms. Livest Sci 145:62–72

Kijlstra A, Eijck IAJM (2006) Animal health in organic livestock production systems: a review. NJAS-Wageningen J Life Sci 54(1):77–94

Lensink BJ, Boivin X, Pradel P, Le Neindre P, Veissier I (2000a) Reducing veal calves' reactivity to people by providing additional human contact. J Anim Sci 78(5):1213–1218

Lensink BJ, Fernandez X, Boivin X, Pradel P, Le Neindre P, Veissier I (2000b) The impact of gentle contacts on ease of handling, welfare, and growth of calves and on quality of veal meat. J Anim Sci 78(5):1219–1226

Lund V (2006) Natural living—a precondition for animal welfare in organic farming. Livest Sci 100(2–3):71–83

Lund V, Algers B (2003) Research on animal health and welfare in organic farming—a literature review. Livest Prod Sci 80(1–2):55–68

Ma S, Joachim S (2006) Review of history and recent development of organic farming worldwide. Agric Sci China 5(3):169–178

Mee JF, Berry DP, Cromie AR (2008) Prevalence of, and risk factors associated with, perinatal calf mortality in pasture-based Holstein–Friesian cows. Animal 2(04):613–620

Ministry of Agriculture (2002–2003) St. meld. nr. 12 (2002–2003) Om dyrehold og dyrevelferd [in Norwegian]. Available at http://www.regjeringen.no/nb/dep/lmd/dok/regpubl/stmeld/20022003/stmeld-nr-12-2002-2003-.html?id=196533. Accessed 23 July 2012

Norwegian Agricultural Authority (2010) Produksjon og omsetning av økologiske landbruksvarer. Rapport for 1. halvår [in Norwegian]. Available at http://www.slf.dep.no/iKnowBase/Content/11783/%C3%98KOLOGISK_RAPPORT_1_HALV%C3%85R_2009.PDF. Accessed 23 July 2012

Norwegian Food Safety Authority (2009) Veileder til forskrift om økologisk produksjon og merking av økologiske landbruksprodukter og næringsmidler, av 4. oktober 2005 nr. 1103. Veileder B. Utfyllende informasjon om økologisk landbruksproduksjon [in Norwegian]. Available at http://www.debio.no/_upl/veileder_b.pdf. Accessed 23 July 2012

Østerås O, Gjestvang M, Vatn S, Sølverød L (2007) Perinatal death in production animals in the Nordic countries—incidence and costs. Acta Vet Scand 49(Suppl 1):S14

Rigby D, Cáceres D (2001) Organic farming and the sustainability of agricultural systems. Agric Syst 68(1):21–40

Rushen J, Taylor AA, de Passillé AM (1999) Domestic animals' fear of humans and its effect on their welfare. Appl Anim Behav Sci 65(3):285–303

Siegford JM, Bernardo TM, Malinowski RP, Laughlin K, Zanella AJ (2005) Integrating animal welfare into veterinary education: using an online, interactive course. J Vet Med Educ 32(4):497–504

Simensen E (2004) Animal care—the human factor in animal production. Norsk Vet 116(1):5–10 [in Norwegian]

Sundrum A (2001) Organic livestock farming: a critical review. Livest Prod Sci 67(3):207–215

Sundrum A, Padel S, Arsenos G, Kuzniar A, Henriksen BIF, Walkenhorst M, Vaarst M (2006) Current and proposed EU legislation on organic livestock production, with a focus on animal health, welfare and food safety: a review. In: Proceedings of the 5th SAFO Workshop, Odense, Denmark. pp 75–90

The Cattle Site (2009) Reducing Calf Mortality. Available at http://www.thecattlesite.com/articles/2224/reducing-calf-mortality. Accessed 23 July 2012

Vaarst M (1998) Omlægning til økologisk drift set fra konsulenters og dyrlægers synsvinkel [In Danish]. Sundhed, velfærd og medicinanvendelse ved omlægning til økologisk drift [Animal health, welfare and medicine use related to the transition to organic milk production. In Danish], Research Centre Foulum Report 699, chapter 2, pp 15–46

Vaarst M, Alban L, Mogensen L, Milan S, Kristensen ES (2001) Health and welfare in Danish dairy cattle in the transition to organic production: problems, priorities and perspectives. J Agric Environ Ethics 14(4):367–390

Vaarst M, Padel S, Arsenos G, Sundrum A, Kuzniar A, Walkenhorst M, Grøva L, Henriksen BIF (2006) Challenges for animal health and welfare in the implementation of the EU legislation on organic livestock production: analysis of questionnaire survey among SAFO participants. In: Rymer C, Vaarst M, Padel S (eds) The 5th SAFO Workshop, Odense, Denmark, 2006. Fischer TaschenbuchVerlag-Forum Wissenschaft Hochschule, pp 43–74

Vaarst M, Padel S, Younie D, Hovi M, Sundrum A, Rymer C (2008) Animal health challenges and veterinary aspects of organic livestock farming identified through a 3 year EU network project. Open Vet Sci J 2:111–116

Vaarst M, Winckler C, Roderick S, Smolders G, Ivemeyer S, Brinkmann J, Mejdell CM,Whistance LK, Nicholas P, Walkenhorst M, Leeb C, March S, Henriksen BIF, Stöger E, Gratzer E, Hansen B, Huber J (2011) Animal health and welfare planning in organic dairy cattle farms. Open Vet Sci J 5(Suppl 1: M5):19–25. Available at http://benthamscience.com/open/tovsj/articles/V005/SI0001TOVSJ/19TOVSJ.pdf

Worthington V (1998) Effect of agricultural methods on nutritional quality: a comparison of organic with conventional crops. Altern Ther Health Med 4(1):58–69

Using qualitative behaviour assessment to explore the link between stockperson behaviour and dairy calf behaviour

CrossMark

Kristian Ellingsen [a],[*], Grahame J. Coleman [b], Vonne Lund [a],[1], Cecilie M. Mejdell [a]

[a] Norwegian Veterinary Institute, PO Box 750 Sentrum, N-0106 Oslo, Norway
[b] The University of Melbourne, Melbourne 3010, VIC, Australia

ABSTRACT

Dairy farming usually implies close and frequent contact between the stockperson and the animals. A good human–animal relationship (HAR) is therefore essential for good animal welfare. To fully understand the quality of the HAR both the stockperson behaviour and the animals' reaction to the handler needs to be assessed, as they mutually affect each other. Qualitative behaviour assessment (QBA) has during the last decade become a method to assess animal welfare through scrutiny of animal body language. The application of this method to characterize stockperson behaviour, on the other hand, is novel. This study aimed to, through the use of QBA, to characterize stockperson behaviour and to portray the body language dairy calves of the animals in his/her care. Further, the study tested the relationships between stockperson behaviour and calf behaviour using structural equation modelling (SEM). The assessments were performed in 2006–2008 on 110 Norwegian dairy farms. The stockperson sample consisted of 79.6% males and 20.4% females, with a mean age of 46 years. The dairy calves (including young stock) were mostly Norwegian Red and were 3 to 298 days old at the day of observation. Ten items of the stockperson QBA were analysed through Principal component analysis. The handling styles that emerged were termed calm/patient, dominating/aggressive, positive interactions and insecure/nervous. The 31 items of the calf QBA were also analysed using principal component analysis and revealed two dimensions of calf behaviour labelled pos/neg mood and high/low arousal. Based on the expected relationships between stockperson behaviour and calf behaviour a structural model was developed and tested using SEM. The analysis revealed that stockpersons who handle their calves patiently and pet and calmly talk to them during handling have animals with higher levels of positive mood, as characterized by high scores on QBA items like friendly and content. Stockpersons with a nervous handling style, or who were dominating and aggressive, on the other hand, had calves with more negative mood. These findings are important as they show the direct link between human behaviour and calf behaviour and once again confirm the significance of good stockmanship. The results also highlight the importance of proper training and self-awareness for those working with livestock.

© 2014 The Authors. Published by Elsevier B.V. All rights reserved.

1. Introduction

1.1. Human–animal relationship

In dairy farming, the stockperson is in frequent and close contact with his/her animals during procedures such as

* Corresponding author. Tel.: +47 91 70 29 70/+47 23 21 63 77.
 E-mail address: Kristian.Ellingsen@vetinst.no (K. Ellingsen).
[1] Sadly passed away during the course of the project.

milking, cleaning and inspection. This is especially true for a country like Norway where production units are small (average dairy herd size is in 2013 was 24 cows (Statistics-Norway, 2013) and animals are kept indoors most of the year.

A good human–animal relationship (HAR), here defined as "the degree of relatedness or distance between the animal and the human, i.e., the mutual perception, which develops and expresses itself in their mutual behaviour" (Estep and Hetts, 1992, p. 6) is therefore fundamental to good animal welfare. A vast number of publications have been dedicated to the topic of HAR in various species, including companion (e.g. Marinelli et al., 2007; Ellingsen et al., 2010) and productions animals (e.g. Coleman et al., 1998; Waiblinger et al., 2002; Breuer et al., 2003). What is generally found, is that animals having a positive bond with their caretaker are safer and easier to handle, while lack of habituation to people, as well as negative handling with shouting and hitting leads to poorer animal welfare, more fear, acute and chronic stress (Hemsworth et al., 2000; Hemsworth, 2003; Simensen, 2004) and reduced reproduction (Hemsworth et al., 1986). Studies have also shown that a negative HAR leads to decreased milk yield and increased residual milk in dairy cattle (Rushen et al., 1999; Waiblinger et al., 2002). On the other hand, calm touching and talking to cattle during milking leads to higher milk yield (Hemsworth and Coleman, 1998).

It has been known for some time that a major factor influencing the HAR is the nature of the daily interactions between the stockperson and the animal (Hemsworth et al., 1981a,b), as stockperson behaviour determines the animals' reaction towards humans (Waiblinger et al., 2006). During the last decades a great deal of work has therefore been done in the area of HAR and animal welfare assessment in production animal species (e.g. Rushen et al., 1999; Waiblinger et al., 2006; Bertenshaw et al., 2008; Windschnurer et al., 2008; Welfare Quality, 2009). In this process a method called qualitative behaviour assessment (QBA) has undergone extensive testing and is proving a time efficient and valid addition to a number of these animal welfare assessment protocols.

1.2. Qualitative behaviour assessment

QBA is an integrated assessment of the whole animal where the animal's body language is evaluated as an indication of the animal welfare state (Wemelsfelder and Lawrence, 2001). Originally the QBA was developed by the use of spontaneous judgements in a process called Free Choice Profiling. Untrained personnel were asked to observe animals for a period of time and then write down the behaviours or mental states they felt best described the animals' status. The observers showed high agreement and the method had good repeatability and correlated well with other behavioural and physiological measures of animal welfare (Wemelsfelder and Lawrence, 2001). The scale was then further developed to a pre-fixed list of descriptors containing words like happy, content, nervous, frustrated and aggressive, as seen in Welfare Quality® (Wemelsfelder et al., 2009a). The QBA has been validated on a wide range of species including veal cattle

and calves, dairy cattle (Rousing and Wemelsfelder, 2006; Wemelsfelder et al., 2009a), horses (Napolitano et al., 2008), pigs (Wemelsfelder et al., 2001) and dairy buffaloes (Napolitano et al., 2012). Using QBA to describe stockperson behaviour, however, is a novel way of characterizing handling styles.

1.3. Aims

Using QBA on stockperson behaviour, this study aimed to characterize different handling styles of stockpersons interacting with their dairy calves and young stock. Using Qualitative Behaviour Assessment on the dairy calves, we also set out to portray the body language of the animals. Haskell et al. (2003) suggested the use QBA to evaluate the response of dairy cows to humans and Brscic et al. (2009) stated that QBA may be sensitive to the quality of human contact. The final aim of the study was therefore, using structural equation modelling (SEM), to develop and test a model showing how stockperson behaviour correlates with the behaviour of the animals.

2. Materials and methods

2.1. Data collection

The current study is based on qualitative behaviour assessment of stockperson and dairy calves, including young stock up to 10 months of age, conducted on 110 Norwegian dairy farms between January 2006 and March 2008. All behaviour registrations were carried out by the same observer, an experienced livestock inspector and agricultural advisor. Farms were randomly selected from a list of dairy producers covering pre-defined regions of Southern Norway. All selected farms were members of the Norwegian Cattle Health Recording System (NCHRS). NCHRS commenced nationally in 1975 (Østerås et al., 2007) to guide farmers in management related issues, including feeding and breeding. Membership is not mandatory, but 98.5% of the Norwegian dairy herds regularly report milk yield, disease occurrence and treatment of individual animals (Tine, 2012). The stockperson that participated was the one who on a daily basis managed the farm's calves and young stock.

The stockperson sample consisted of 88 (80.0%) males and 22 (20.0%) female, with a mean age of 46 years (SE ± 0.04). 87 (79.1%) participants were married or had a partner and 58 (52.7%) had children. 13 (11.8%) of the respondents had primary school as their highest level of finished education, 73 (66.4%) had completed upper secondary school and 14 (12.7%) had university college or university degrees. Educational information was missing for 10 (9.1%) of the sample. The stockpersons were generally very experienced with dairy calves, as mean years of experience was 24.5 (SE ± 1.22).

The vast majority of the calves included in the QBA were Norwegian Red. Remaining calves were Norwegian Red cross breeds, Jerseys, Simmental, or the local breeds Norwegian Red Polled Cattle and Blacksided Trønder and Nordland Cattle. The mean number of calves and young stock on the farms that were included in the study was 31

(range 10–120 animals) and their age at the time of the visit varied from 3 to 298 days. At each farm, five calves were observed. The test animals were randomly chosen from a list containing the ear tag numbers of all suitable calves before entering the barn. Some farms had concentrated calving and hence the five animals were approximately the same age. Other farms had spread calving resulting in up to six months age differences in the test animals.

2.2. The qualitative behaviour assessment—Stockperson

The first QBA was performed to determine the behaviour of the stockperson. The stockperson was blind to the purpose of the study and therefore, even though the observer could interfere with the usual farm management, a "true" stockperson management behaviour could be expected to be observed. The stockperson was asked to do a chest measurement on five calves successively, and data is hence based on $5 \times 110 = 550$ interactions. In addition to obtaining calf weights the observer monitored the behaviour of the human in his/her interactions with the calves. After the observation period had finished, the observer scored, from memory, the body language of the stockperson according a list of 17 descriptors on a visual analogue scale (VAS). The descriptors included in the stockperson QBA were: quick, dominating, aggressive, fearful, patient, careful, calm, determined, focused, insecure, careless, talks to the animals, cuddles the animals, inventive, nervous, boisterous and including. After the test had ended, the stockperson was informed of the second objective of the study and was asked for permission to use the data. All 110 participants consented.

2.3. The qualitative behaviour assessment—Calf

The second QBA was carried out to evaluate the behaviour of dairy calves on the 110 farms. According to standard test procedure, the observer studied the animals for 10–20 min and then assessed the animals' behavioural expression by scoring them on a given list of 31 descriptors on a VAS. To avoid further influence from the animals, this was done in another room/section of the barn. The descriptors included in the calf QBA were: nervous, frustrated, fearful, enjoying, distressed, uncomfortable, friendly, content, sociable, uneasy, calm, confident, agitated, unwell, happy, scared, positively occupied, relaxed, boisterous, inquisitive, playful, tense, aggressive, bored, depressed, active, lively, irritable, vigilant, apathetic, indifferent and welfare overall. The terms used in our study were the same terms as used in Welfare Quality®, but as the descriptor "welfare overall" includes non-animal environmental features, this item was excluded from the analyses, as recommended (Wemelsfelder et al., 2009a).

2.4. Statistical analyses

For analysis, the VAS was converted into a 125 mm long line and the distance from the left-hand side of the VAS to the line drawn by the observer was measured, giving the score for that descriptor. To create clearly defined handling styles with optimal factor loading, the stockperson QBA was further analysed using Principal Component Analysis (PCA) with Varimax rotation on 10 descriptors. To comply with the standard way of analysing animal QBAs (e.g. Andreasen et al., 2013; Phythian et al., 2013), all 31 items were included in the calf QBA analysis with no rotation performed. The factor scores of each individual handling style, along with the factor scores of the two dimensions of calf behaviour, were used as separate variables in a structural equation model (SEM) (Byrne, 2010). The suitability of both QBA scales were analysed using the Kaiser–Meyer–Olkin measure of sampling adequacy.

Several fit indices were utilized to evaluate the suitability of the SEM. First, normed Chi square (X^2/df) was chosen over traditional Chi-square statistics (X^2), as it takes into consideration the complexity of the model and is less sensitive to sample size. The normed Chi square should be less than 2 (Schumacker and Lomax, 2004). Second, the Comparative Fit Index (CFI) was used. With this measure, values above 0.90 and 0.95 indicates acceptable and good fit, respectively (Byrne, 2010). Lastly, the Root Mean-Square Error of Approximation (RMSEA) was utilized. RMSEA values of less than 0.05 indicate good fit (Byrne, 2010).

All statistical analyses were performed using SPSS v.20. The SEM was created using AMOS v. 20.

3. Results

3.1. Principal component analysis (PCA)

Prior to performing PCA, the suitability of both scales for factor analysis was assessed. Inspection of the correlation matrix revealed a majority of coefficients of 0.3 and above. The Kaiser–Meyer–Olkin value was 0.77 for the stockperson QBA and 0.89 for the calf QBA, hence both exceeding the recommended value of 0.6. Bartlett's Test of Sphericity reached statistical significance for both scales, supporting the factorability of the correlation matrices.

3.2. The qualitative behaviour assessment—Stockperson

Principal component analysis with extraction of four components explained 25.5%, 24.4%, 18.1% and 16.9% of the variance, respectively. To aid in the interpretation of these four components Varimax rotation was performed (Table 1).

PCA analysis revealed four relevant handling styles termed; calm/patient (PC1), dominating/aggressive (PC2), positive interactions (PC3) and insecure/nervous (PC4). A stockperson who has positive interactions actively engages in contact with the animals, talking calmly and/or touching and petting them. By calm/patient is implied that the stockperson treats the animals in a quiet and careful way, without rushing or stressing them. This handling style does not, however, infer the same degree of closeness or passion as positive interactions. A dominating/aggressive handling style holds that the stockperson is noisy, rowdy and forceful when handling the animals, while the final handling style, insecure/nervous, aims to portray a person who is uncomfortable working with calves and shows anxious or apprehensive behaviour. The two former handling styles

Table 1
The table shows how the 10 stockperson behaviour scores (QBA) are grouped in four handlings styles (PC1–4). The items were analysed using principal component analysis with Varimax rotation.

Item	PC1	PC2	PC3	PC4	Communalities
Calm	**0.914**	−0.210	0.125	−0.046	0.897
Careful	**0.889**	−0.289	0.183	0.104	0.918
Patient	**0.741**	−0.401	**0.366**	−0.035	0.845
Dominating	−0.175	**0.882**	−0.177	−0.138	0.858
Boisterous	**−0.350**	**0.818**	−0.140	0.110	0.823
Aggressive	**−0.373**	**0.717**	−0.038	0.290	0.739
Talks to the animals	0.174	−0.062	**0.892**	0.029	0.832
Cuddles the animals	0.191	−0.184	**0.882**	−0.038	0.849
Insecure	0.096	−0.165	−0.026	**0.916**	0.877
Nervous	−0.093	**0.352**	0.024	**0.845**	0.848

Note: Loadings > ± 0.30 for each item are bolded.

(PC1 and PC3) can be viewed as positive, the two latter (PC2 and PC4) as negative.

3.3. The qualitative behaviour assessment—Calf

Principal components analysis with extraction of two components was performed. The two factors explained 45.4% and 15.7% of the variance. To comply with the standardized way of analysing QBA data, no rotation was performed. A loading plot showing the relationship among the calf QBA descriptors is given in Fig. 1.

PCA analysis revealed two dimensions of calf behaviour labelled positive/negative mood (PC1) and high/low arousal (PC2) (Table 2).

Distinct clusterings along two axes were discovered in the calf QBA data. Animals receiving low scores on PC1 (Mood) have high levels of positive descriptors and low levels of negative descriptors, while animals receiving high scores on the axis have high levels of negative descriptors and low levels of positive descriptors. Mood descriptors can either be high or low in arousal, as reflected by their location relative to the PC2 (Arousal) axis.

3.4. Structural equation modelling

Based on the expected relationships between stockperson behaviour and calf behaviour a structural model was developed and tested using SEM (Fig. 2).

All pathways were significant at the 0.05 level. The first endogenous variable, positive/negative mood, had four direct pathways from exogenous variables. In descending order or magnitude, these pathways reflected the influence of positive interactions ($\beta = -0.41$), calm/patient ($\beta = -0.28$), insecure/nervous ($\beta = 0.25$), and dominating/aggressive ($\beta = 0.23$). Combined these four variables account for 36% of the variance in positive/negative mood within the context of the model. The second endogenous variable, high/low arousal, also had four direct pathways from exogenous variables, reflecting the influence of insecure/nervous ($\beta = -30$), calm/patient ($\beta = -0.25$), dominating/aggressive ($\beta = 0.23$), and positive interactions ($\beta = 0.20$) in descending order of magnitude. Combined these four variables account for 24% of the variance in high/low arousal within the context of the model.

Put differently, results showed that a stockperson who interacts with the animals through gently petting and

Table 2
The table shows how the 31 calf behaviour scores (QBA) are grouped in two dimensions (PC1–2) representing mood and arousal. The items were analysed using Principal component analysis without rotation.

Item	PC1	PC2	Communalities
Nervous	**0.837**	**0.378**	0.664
Frustrated	**0.816**	0.240	0.479
Fearful	**0.816**	0.247	0.658
Enjoying	**−0.814**	0.250	0.726
Distressed	**0.806**	−0.001	0.721
Uncomfortable	**0.805**	−0.100	0.617
Friendly	**−0.803**	0.134	0.192
Content	**−0.793**	**0.402**	0.579
Sociable	**−0.775**	0.157	0.292
Uneasy	**0.773**	**0.345**	0.637
Calm	**−0.761**	−0.010	0.791
Confident	**−0.759**	0.201	0.473
Agitated	**0.756**	**0.386**	0.725
Unwell	**0.745**	−0.026	0.345
Happy	**−0.740**	**0.542**	0.723
Scared	**0.734**	**0.440**	0.662
Positively occupied	**−0.713**	**0.487**	0.279
Relaxed	**−0.689**	−0.068	0.718
Boisterous	**0.676**	**0.506**	0.746
Inquisitive	**−0.666**	0.202	0.555
Playful	**−0.657**	**0.535**	0.735
Tense	**0.620**	0.298	0.732
Aggressive	**0.505**	0.191	0.485
Bored	**0.458**	−0.264	0.631
Depressed	**0.435**	−0.051	0.843
Active	0.063	**0.812**	0.713
Lively	**−0.348**	**0.783**	0.716
Irritable	**0.550**	**0.574**	0.625
Vigilant	**0.556**	**0.573**	0.401
Apathetic	0.291	**−0.562**	0.842
Indifferent	0.265	**−0.524**	0.650

Note: Loadings > ± 0.30 for each item are bolded.

talking calmly have calves with a higher degree of positive mood, as characterized by high scores on QBA items like friendly, content and sociable. The same is true for stockpeople who are calm and patient when interacting with their animals. Stockpersons who have a nervous or insecure handling style, or stockpeople who show dominating or aggressive behaviour in contact with the calves, have more negative mood among the animals, as characterized by high scores on QBA items like nervous, frustrated and fearful.

The fit statistics indicated an almost perfect fit of the model to the data (X^2 (7) = 0.78, $p > 0.05$, $X^2/df = 0.11$, CFI = 1.0, RMSEA = 0.00).
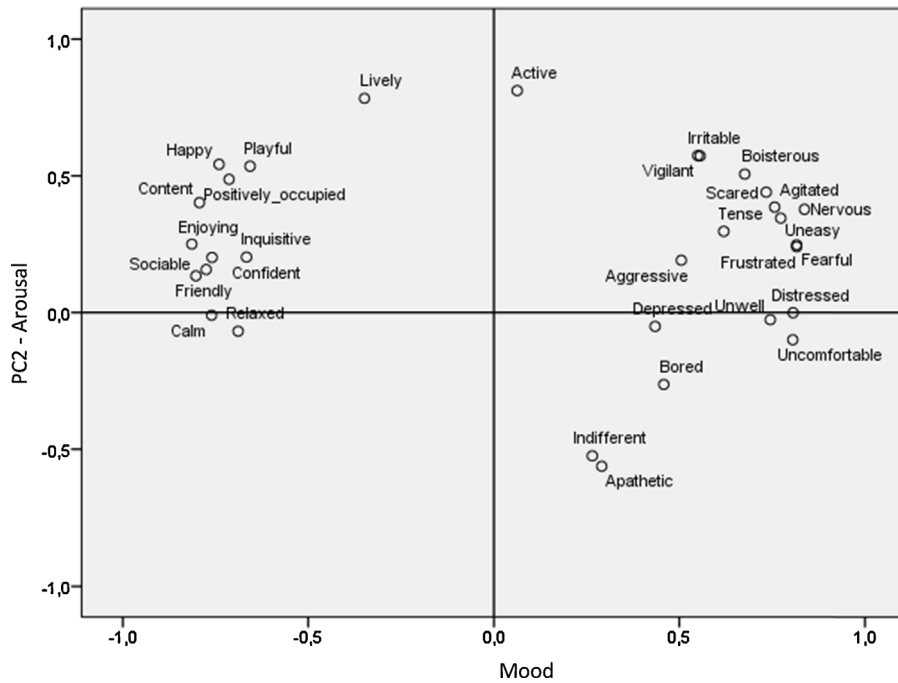
Fig. 1. This 2-dimensional loading plot shows the relationship among the 31 QBA items describing calf behaviour on PC1 (Mood) and PC2 (Arousal). A low score on PC1 indicates positive mood, while a high score indicates negative mood. A low score on PC2 indicates low level s of arousal, while a high score indicates high arousal.
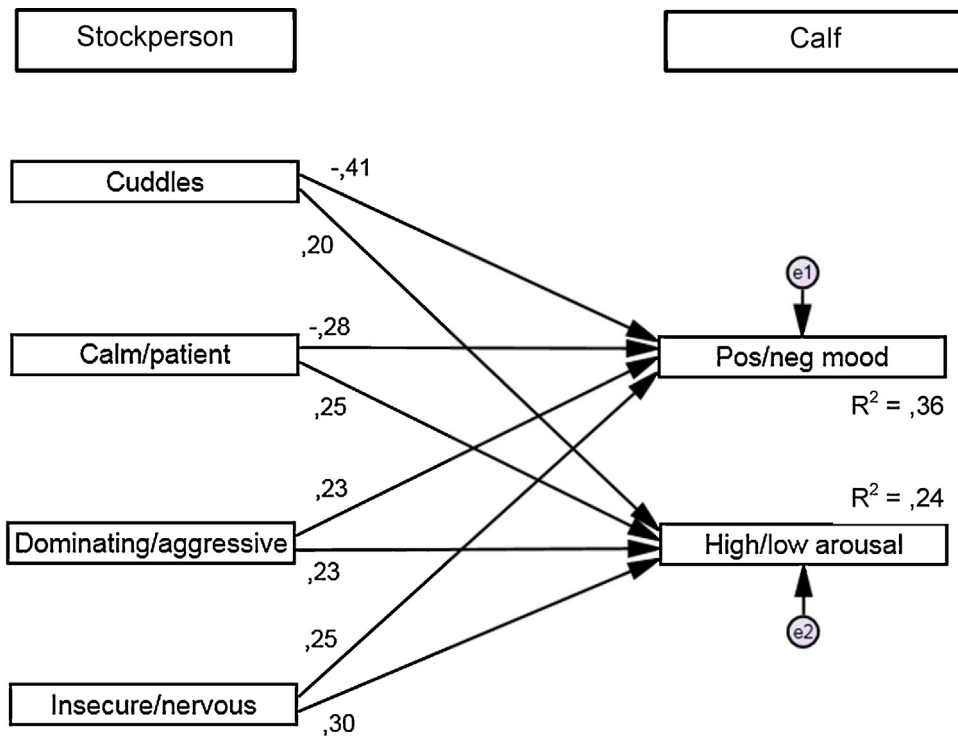

Fig. 2. This figure shows how the four stockperson handling styles (PC1–4) relate to the two dimensions of calf behaviour (PC1–2). A structural equation model with standardized regression weights (B) is used. R^2 values are given for each of the two dependent variables. e1 and e2 denotes measurement error associated with observed variables.

4. Discussion

4.1. Stockperson behaviour

In the current study, four relevant handling styles were identified, termed positive interactions, calm/patient, dominating/aggressive, and insecure/nervous. Other research papers have characterized handler behaviour similar to the dimensions the current study. In 2002, Waiblinger et al. investigated the relationship between attitudes, personal characteristics and behaviour of stockpersons and subsequent behaviour and production of dairy cows. In their study, positive stockperson behaviour was used as a collective term to describe handlers who talked quietly, petted or touched the cattle (Waiblinger et al., 2002). (Lensink et al., 2000, 2001) also used 'positive farmers' contacts with calves' to characterize farmers who petted, touched, and talked to the calves in a friendly manner. Negative or aversive stockperson behaviour is often characterized by hitting, slapping and loud vocalizations (e.g. Munksgaard et al., 1997; Waiblinger et al., 2002). No forceful tactile interactions were observed in the current study.

4.2. Calf behaviour

Two dimensions of calf behaviour, positive/negative mood and high/low arousal, were detected in the current analyses. Variations of these dimensions are commonly seen in QBA studies. Following Free Choice Profiling and generalized procrustes analysis (GPA), Rousing and Wemelsfelder (2006) found two main dimensions associated with social behaviour expression in dairy cattle. The first dimension was characterized as relaxed/calm versus aggressive/bullying and the second as passive/indifferent versus playful/sociable. Reliable clustering along two dimensions was also reported by Wemelsfelder et al. (2009a), based on the same QBA items as the current study. Looking at QBA data for dairy cattle, beef bulls and veal calves, the authors reported that one dimension distinguished between positive and negative mood, while the other dimension discriminated between high and low levels of arousal in these moods (Wemelsfelder et al., 2009a). An Italian study looking to integrate QBA with clinical/health protocols in veal calves also found one dimension associated with positive and negative mood descriptors, while the other dimension related to activity and boredom (Brscic et al., 2009). In yet another recent study, Andreasen et al. (2013) two QBA dimensions were also identified, one characterized by calm/relaxed to uneasy/agitated, the other by indifferent/distressed to lively/playful. Similar dimensions are also found in QBA studies on pigs (Wemelsfelder et al., 2001; Rutherford et al., 2012) and sheep (Phythian et al., 2013).

4.3. The effect of stockperson behaviour on calf behaviour

Our results support the previously recognized relationship between stockperson handling style and calf mood and level of arousal. Waiblinger et al. (2006) state that "the stockpersons' behaviour is a major variable determining animals' fear of or confidence in human beings and, hence, the quality of the HAR". It is well established that cows (Munksgaard et al., 1997) and dairy calves (de Passille et al., 1996) can discriminate between handlers based on treatment, as seen by avoidance behaviour. Cows that experience a high percentage of positive interactions (talking quietly, petting and touching) and low percentage of negative interactions (forceful use of stick or hand, shouting and impatient talk) with handlers in the milking parlour, were found to avoid humans less (Waiblinger et al., 2002). Cows, however, kept a greater distance to the handler, as well as urinated and defecated more frequently, following aversive treatment (striking the cow forcefully with open hand) (Munksgaard et al., 1997). Lower levels of withdrawal is also associated with positive contact (petting, touching, talking in a friendly manner) between calves and handlers, as shown by Lensink et al. (2001). Hemsworth and Coleman (1998) have shown that withdrawal is associated with fear in the animals, and behaviour by the stockpeople causing withdrawal is hence associated with poor animal welfare. Our findings confirm these results. A high score on QBA descriptors like tense, fearful, scared and nervous, loading high on the negative mood dimension is therefore associated with aggressive/dominating as well as insecure/nervous handlers. Descriptors like confident, calm, and friendly, on the other hand, loading high on the positive mood dimension, is associated with handlers who are calm/patient and touches and talks to the calves.

A link between insecure and nervous handlers and tense and fearful animals has also been suggested in horses (Hallman and Demmin, 2005). Fear and nervousness in animals is associated with stress and reduced animal welfare (Rushen et al., 1999). In addition, nervous animals are more unpredictable and unsafe to handle, hence increasing the risk of injury to themselves or the stockperson (Hemsworth and Coleman, 1998; Rushen et al., 1999). Waiblinger et al. (2006) also suggest that a negative feedback cycle might be established between the animals and their caretaker whereby the attitudes and behaviour of the handler worsens with subsequent increases in fear of humans among the animals. This could perhaps also be the case in our sample. If handlers who are insecure/nervous experience more negative mood in their herds, they may feel the need to use dominating/aggressive behaviour to control the animals.

In accordance with current results, a number of studies have also found stockperson behaviour to influence the level of arousal in the animals. The use of negative tactile interactions, loud harsh vocalisations and high speed of movement among the handlers when moving cows have been found to be positively correlated with restlessness in the animals (Breuer et al., 2000). Waiblinger et al. (2002) also suggest that positive, calming interactions might reduce the activity level in cattle. This was not supported in the current study as all four handling styles were positively related to high arousal in the animals. A reason for this may be that the two studies above were conducted on adult cattle, while our observations were based on calves. Grown cattle spend about 5–8 h ruminating and rest lying for about 10–12 h per 24 h (Ekesbo, 2011), implying that low activity levels are desirable. The

calves in our sample, on the other hand, were aged between 1 and 9 months, meaning that more play behaviour can be expected (Bekoff, 2001). It is also likely that calves are more easily aroused by handlers, in contrast to adult cattle that have been habituated to humans over several years. According to Wemelsfelder et al. (2009a), arousal does not directly influence welfare. The dimension instead has an important function in giving a meaningful transition between positive and negative mood on the first dimension, and hence adds to the information on animal welfare given by the mood dimension.

4.4. The validity of qualitative behaviour assessment

During recent years, QBA has been used to evaluate cattle welfare, mood and behaviour in a number of ways, including pre-slaughter behaviour in Angus steers (Stockman et al., 2012), stress during transport (Stockman et al., 2011) and social behaviour in dairy cows (Rousing and Wemelsfelder, 2006). More and more papers are also being published correlating QBA with physiological measures (Stockman et al., 2011, 2012; Rutherford et al., 2012; Wickham et al., 2012) and suggesting that the method can detect subtle differences equal to or beyond what quantitative measures can detect (Wemelsfelder et al., 2001). Superior ability to pick up small changes between herds was also one potential explanation for why Andreasen et al. (2013) failed to find meaningful relationships between QBA scores and other Welfare Quality® measures (see Andreasen et al., 2013 for discussion). Two studies published in 2009 concluded that rearing environment for pigs (Wemelsfelder et al., 2009b) and veal calves (Brscic et al., 2009) did not distort observer characterization of behaviour expression. It has also been shown that diverging backgrounds, experience and views do not have negative effect on inter- or intra-observer reliability (Wemelsfelder et al., 2012). The use of QBA as a measure of welfare on production animals has hence been validated by those groups.

Conversely, QBA of stockperson behaviour is novel and has so far not been validated. It is impossible without validation to know how e.g. attitudes, demographics and societal norms influence stockperson QBA scores. This uncertainty is the reason why stockperson QBA was analysed differently than the calf QBA. While the latter was analysed in accordance with other QBA studies (all items, no rotation), well-defined and relevant handling styles were created through Varimax rotation of certain QBA descriptors in order to optimize factor loadings. Based on the significant associations also found in other studies, our study has shown promising potential of the stockperson QBA to predict animal behaviour.

5. Conclusions and implications

Our findings suggest that human and animal behaviour are closely linked. This underlines the significance of good stockmanship. Not only proper education of stockpersons but also awareness of one's own behaviour is essential for those working with livestock. The knowledge generated in this study also allows us to tailor attitude and behaviour change interventions to stockpersons, which in turn may cause advancements in the HAR and ultimately lead to a higher level of animal welfare.

References

Andreasen, S.N., Wemelsfelder, F., Sandøe, P., Forkman, B., 2013. The correlation of qualitative behavior assessments with welfare quality® protocol outcomes in on-farm welfare. assessment of dairy cattle. Appl. Anim. Behav. Sci. 143, 9–17.
Bekoff, M., 2001. Social play behaviour. Cooperation, fairness, trust, and the evolution of moralit. J.Conscious. Stud. 8, 81–90.
Bertenshaw, C., Rowlinson, P., Edge, H., Douglas, S., Shiel, R., 2008. The effect of different degrees of 'positive' human–animal interaction during rearing on the welfare and subsequent. Production of commercial dairy heifers. Appl. Anim. Behav. Sci. 114, 65–75.
Breuer, K., Hemsworth, P.H., Barnett, J.L., Matthews, L.R., Coleman, G.J., 2000. Behavioural response to humans and the productivity of commercial dairy cows. Appl. Anim. Behav. Sci. 66, 273–288.
Breuer, K., Hemsworth, P.H., Coleman, G.J., 2003. The effect of positive or negative handling on the behavioural and physiological responses of nonlactating heifers. Appl. Anim. Behav. Sci. 84, 3–22.
Brscic, M., Wemelsfelder, F., Tessitore, E., Gottardo, F., Cozzi, G., Van Reenen, C.G., 2009. Welfare assessment: correlations and integration between a qualitative behavioural assessment and a clinical/health protocol applied in veal calves farms. Ital. J.Anim. Sci. 8, 601–603.
Byrne, B.M., 2010. Structural Equation Modeling With AMOS: Basic Concepts, Applications and Programming, second ed. Hoboken, NJ, Taylor and Francis.
Coleman, G.J., Hemsworth, P.H., Hay, M., 1998. Predicting stockperson behaviour towards pigs from attitudinal and job-related variables and empathy. Appl. Anim. Behav. Sci. 58, 63–75.
de Passille, A.M., Rushen, J., Ladewig, J., Petherick, C., 1996. Dairy calves' discrimination of people based on previous handling. J. Anim. Sci. 74, 969–974.
Ekesbo, I., 2011. Farm Animal Behaviour: Characteristics for Assessment of Health and Welfare. CABI, Wallingford.
Ellingsen, K., Zanella, A.J., Bjerkås, E., Indrebø, A., 2010. The relationship between empathy, perception of pain and attitudes toward pets among Norwegian dog owners. Anthrozoôs 23, 231–243.
Estep, D.Q., Hetts, S., 1992. Interactions relationships and bonds: the conceptual basis for scientist-animal relations. In: Davis, H., Balfour, D. (Eds.), The Inevitable Bond: Examining Scientist–Animal Interactions. Cambridge University Press, Cambridge; New York, NY, pp. 6–26.
Hallman, E., Demmin, D.M., 2005. Animal handling safety. In: National AG Safety Database.
Haskell, M.J., Rennie, L.J., Bowell, V.A., Wemelsfelder, F., Lawrence, A.B., 2003. On-farm assessment of the effect of management and housing type on behaviour and welfare in dairy cattle. Anim. Welfare 12, 553–556.
Hemsworth, P.H., 2003. Human-animal interactions in livestock production. Appl. Anim. Behav. Sci. 81, 185–198.
Hemsworth, P.H., Barnett, J.L., Hansen, C., 1981a. The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. Horm. Behav. 15, 396–403.
Hemsworth, P.H., Barnett, J.L., Hansen, C., 1986. The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. Appl. Anim. Behav. Sci. 15, 303–314.
Hemsworth, P.H., Brand, A., Willems, P., 1981b. The behavioural response of sows to the presence of human beings and its relation to productivity. Livest. Prod. Sci. 8, 67–74.
Hemsworth, P.H., Coleman, G.J., 1998. Human–Livestock Interactions: The Stockperson and the Productivity of Intensively Farmed Animals. CAB International, Wallingford, UK.
Hemsworth, P.H., Coleman, G.J., Barnett, J.L., Borg, S., 2000. Relationships between human–animal interactions and productivity of commercial dairy cows. J. Anim. Sci. 78, 2821–2831.

Lensink, B.J., Veissier, I., Florand, L., 2001. The farmers' influence on calves' behaviour, health and production of a veal unit. Anim. Sci. 72, 105–116.

Lensink, J., Biossy, A., Veissier, I., 2000. The relationship between farmers' attitude and behaviour towards calves, and productivity of veal units. Ann. Zootech. 49, 313–327.

Marinelli, L., Adamelli, S., Normando, S., Bono, G., 2007. Quality of life of the pet dog: influence of owner and dog's characteristics. Appl. Anim. Behav. Sci. 108, 143–156.

Munksgaard, L., De Passille, A.M., Rushen, J., Thodberg, K., Jensen, M.B., 1997. Discrimination of people by dairy cows based on handling. J. Dairy Sci. 80, 1106–1112.

Napolitano, F., De Rosa, G., Braghieri, A., Grasso, F., Bordi, A., Wemelsfelder, F., 2008. The qualitative assessment of responsiveness to environmental challenge in horses and ponies. Appl. Anim Behav. Sci. 109, 342–354.

Napolitano, F., De Rosa, G., Grasso, F., Wemelsfelder, F., 2012. Qualitative behaviour assessment of dairy buffaloes (*Bubalus bubalis*). Appl. Anim. Behav. Sci. 141 (3–4), 91–100.

Phythian, C., Michalopoulou, E., Duncan, J., Wemelsfelder, F., 2013. Inter-observer reliability of qualitative behavioural assessments of sheep. Appl. Anim. Behav. Sci. 144, 73–79.

Rousing, T., Wemelsfelder, F., 2006. Qualitative assessment of social behaviour of dairy cows housed in loose housing systems. Appl. Anim. Behav. Sci. 101, 40–53.

Rushen, J., Taylor, A.A., de Passillé, A.M., 1999. Domestic animals' fear of humans and its effect on their welfare. Appl. Anim. Behav. Sci. 65, 285–303.

Rutherford, K.M.D., Donald, R.D., Lawrence, A.B., Wemelsfelder, F., 2012. Qualitative behavioural assessment of emotionality in pigs. Appl. Anim. Behav. Sci. 139, 218–224.

Schumacker, R.E., Lomax, R.G., 2004. A Beginner's Guide to Structural Equation Modeling, second ed. Lawrence Erlbaum Associates, Inc., New Jersey.

Simensen, E., 2004. Animal care—the human factor in animal production. Norsk Veterinærtidsskrift [in Norwegian] 116, 5–10.

Statistics-Norway, 2013. Husdyrhald, 1. januar 2013, førebelse tal, https://www.ssb.no/jord-skog-jakt-og-fiskeri/statistikker/jordhus, Accessed 02.02.2014.

Stockman, C.A., Collins, T., Barnes, A.L., Miller, D., Wickham, S.L., Beatty, D.T., Blache, D., Wemelsfelder, F., Fleming, P.A., 2011. Qualitative behavioural assessment and quantitative physiological measurement of cattle naïve and habituated to road transport. Anim. Prod. Sci. 51, 240–249.

Stockman, C.A., McGilchrist, P., Collins, T., Barnes, A.L., Miller, D., Wickham, S.L., Greenwood, P.L., Cafe, L.M., Blache, D., Wemelsfelder, F., Fleming, P.A., 2012. Qualitative behavioural assessment of angus steers during pre-slaughter handling and relationship with temperament and physiological responses. Appl. Anim. Behav. Sci 142, 125–133.

Tine, 2012. Tine Rådgiving Statistikksamling 2012. In: Roalkvam, T. (Ed.), https://medlem.tine.no/cms/om-oss/statistikker/_attachment/297302?_ts=13d92495db0.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A.M., Visser, E.K., Jones, R.B., 2006. Assessing the human–animal relationship in farmed species: a critical review. Appl. Anim Behav. Sci. 101, 185–242.

Waiblinger, S., Menke, C., Coleman, G., 2002. The relationship between attitudes, personal characteristics and behaviour of stockpeople and subsequent behaviour and production of dairy cows. Appl. Anim. Behav. Sci. 79, 195–219.

Welfare Quality, 2009. An overview of the development of the welfare quality assessment systems. In: Keeling, L. (Ed.), Welfare Quality Reports No. 12. Cardiff University, UK.

Wemelsfelder, F., Hunter, A.E., Paul, E.S., Lawrence, A.B., 2012. Assessing pig body language: agreement and consistency between pig farmers, veterinarians, and animal activists. J. Anim Sci. 90, 3652–3665.

Wemelsfelder, F., Hunter, T.E.A., Mendl, M.T., Lawrence, A.B., 2001. Assessing the 'whole animal': a free choice profiling approach. Anim. Behav. 62, 209–220.

Wemelsfelder, F., Lawrence, A.B., 2001. Qualitative assessment of animal behaviour as an on-farm welfare-monitoring tool. Acta Agric. Scand. Sect. A 51, 21–25.

Wemelsfelder, F., Millard, F., Rosa, G.D., Napolitano, F., 2009a. Qualitative behaviour assessment. In: Forkman, B., Keeling, L. (Eds.), Assessment of Animal Welfare Measures for Dairy Cattle, Beef Bulls and Veal Calves. Cardiff University, UK, pp. 215–224.

Wemelsfelder, F., Nevison, I., Lawrence, A.B., 2009b. The effect of perceived environmental background on qualitative assessments of pig behaviour. Anim. Behav. 78, 477–484.

Wickham, S.L., Collins, T., Barnes, A.L., Miller, D.W., Beatty, D.T., Stockman, C., Blache, D., Wemelsfelder, F., Fleming, P.A., 2012. Qualitative behavioral assessment of transport-naïve and transport-habituated sheep. J. Anim. Sci. 90, 4523–4535.

Windschnurer, I., Schmied, C., Boivin, X., Waiblinger, S., 2008. Reliability and inter-test relationship of tests for on-farm assessment of dairy cows' relationship to humans. Appl. Anim. Behav. Sci. 114, 37–53.

Østerås, O., Solbu, H., Refsdal, A.O., Roalkvam, T., Filseth, O., Minsaas, A., 2007. Results and evaluation of thirty years of health recordings in the norwegian dairy cattle population. J. Dairy Sci. 90, 4483–4497.
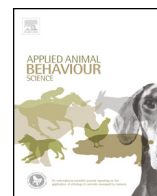
The effect of physical contact between dairy cows and calves during separation on their post-separation behavioural response

Julie Føske Johnsen [a],[*], Kristian Ellingsen [a], Ann Margaret Grøndahl [a], Knut Egil Bøe [b], Lena Lidfors [c], Cecilie Marie Mejdell [a]

[a] *Norwegian Veterinary Institute, Department of Health Surveillance, Postbox 750 Sentrum, 0106 Oslo, Norway*
[b] *Norwegian University of Life Sciences, Department of Animal and Aqua Cultural Sciences, P.O. Box 5003, 1432 Aas, Norway*
[c] *Swedish University of Agricultural Sciences, Department of Animal Environment and Health, P.O. Box 234, 532 23 Skara, Sweden*

ARTICLE INFO

ABSTRACT

Premature breaking of the maternal bond between a cow and her calf triggers a strong behavioural response which renders separation and weaning major welfare challenges in suckling systems. Fence-line separation and weaning allowing physical contact has been evaluated for beef cattle and is claimed to reduce stress at separation. The objective of this study was to compare the post-separation behavioural response of cow–calf pairs separated either with a fence-line (FL, $n = 8$ pairs) allowing physical contact or with a solid wall (SW, $n = 8$ pairs) allowing merely auditory contact. After separation, all calves were offered milk (2.0 L, thrice a day) from a teat bottle to reduce the effect of hunger. Behaviours were recorded for 4 h at days 0 (day of separation), 1, 2, 3 and 4 from which we calculated the total behavioural reaction (area under the curve, AUC). Treatment differences were analysed with two samples Wilcoxon test. Most of the post-separation responses occurred during day 0–2. Results show that FL calves performed less high-pitched (open mouthed) vocalizations as compared to SW calves ($P = 0.003$). Median number of calls/4 h and range for FL vs. SW calves respectively were: *day 0*; 13 (0–94) vs. 361 (2–658), *day 1*; 10 (1–247) vs. 274 (18–872), *day 2*; 0 (0–4) vs. 48 (0–365), *day 3*; 0 (0–1) vs. 4 (0–135) and *day 4*; 0 (0–2) vs. 18 (0–84). Calves separated with FL also showed a lower frequency than SW calves ($P = 0.003$) of alert behaviour defined as high-head posture, pointed ears with focus towards the cow (median percentage of observations and range for FL and SW calves respectively): *day 0*; 23 (0–50) vs. 26 (0–50), *day 1*; 3 (0–12) vs. 12 (4–36), *day 2*; 0 (0–6) vs. 12 (0–18), *day 3*; 0 (0–2) vs. 3 (0–18) and *day 4*; 0 (0–2) vs. 15 (0–28). No significant treatment differences were found for other calf behaviours or in the post-separation behavioural responses of the cow. This is the first study to quantitatively assess two different vocalizations in response to separation; low-pitched vocalizations (with closed mouth) and high-pitched vocalizations in response to separation. Recordings of high-pitched vocalization seem to be a promising tool for assessing distress at separation. In conclusion, FL separation allowing physical contact may reduce the vocal response and alert behaviour of calves.

© 2015 Elsevier B.V. All rights reserved.

* Corresponding author. Tel.: +47 23 21 64 47/95 49 61 60; fax: +47 23 21 63 01.
E-mail addresses: Julie.johnsen@vetinst.no (J.F. Johnsen), Kristian.Ellingsen@vetinst.no (K. Ellingsen), Ann-Margaret.grondahl@vetinst.no (A.M. Grøndahl), Knut.boe@nmbu.no (K.E. Bøe), Lena.Lidfors@slu.se (L. Lidfors), Cecilie.mejdell@vetinst.no (C.M. Mejdell).

1. Introduction

Under natural conditions, the cow and calf remain together until the calf is gradually weaned at approximately 8–11 months (Reinhardt and Reinhardt, 1981). In conventional dairy practice, the calf is separated from the dam immediately after birth. Calves raised in organic dairy production, a production system emphasizing animal welfare and natural behaviour (IFOAM), are mandated to suckle their dam for only a few days: 3 days in Norway (Debio, 2005) and at least 24 h in Sweden and Denmark (Krav, 2012; Vidensenteret for økologisk landbrug, 2012). It has been found that the early separation is perceived by the public to be unnatural and problematic for the welfare of cow and calf, e.g. Ventura et al. (2013). Some organic dairy farmers practice cow–calf suckling for an extended period, mostly for some weeks.

When mutually strongly bonded mothers and offspring are separated prematurely, bouts of reinstatement behaviour such as locomotion (searching) and vocal signalling are performed by both, interspersed by periods of energy-conserving depression (Panksepp, 1998). The vocalizations of cattle have been described by Kiley (1972), Weary and Chua (2000) and Padilla de la Torre et al. (2015): short and soft vocalizations, often performed with the mouth closed, are used for communication over short distances, whereas open-mouthed, long and loud calls are used when isolated or separated under natural conditions. However, no studies have quantifiably assessed the different types of vocalizations in response to forced separation.

After an extended or even short suckling period, separation and weaning are major welfare challenges (Flower and Weary, 2001). Many studies have been dedicated to developing alternative separation methods reducing the behavioural reactions. One such method is fence-line (FL) separation. Originally developed for beef cattle, this separation method allows the dam and her offspring to have visual and some tactile contact over a fence during the days following separation (Nicol, 1977). Results from studies utilizing this separation method are, however, contradictory. Some studies have found FL separated cows and calves to vocalize and walk less compared to those that are abruptly and totally separated, indicating lower levels of stress (Nicol, 1977; Stookey et al., 1997; Price et al., 2003). Other studies have, on the contrary, found physical contact after separation to cause a more stressful separation than no contact (Solano et al., 2007; Enriquez et al., 2010). Stěhulová et al. (2008) showed that dairy cows separated with only visual and auditory (but no physical) contact with the calf, showed a much stronger behavioural response compared to cows housed without any contact with their calves after separation.

One evaluated weaning method is two-step weaning, where calves are kept with their dams but prevented from suckling by a nose flap for some days before they are separated from the dam (Haley, 2006; Loberg et al., 2008; Enriquez et al., 2010). This means that the calves have stopped suckling before they are separated from their dam. When the calves are not weaned off milk before separation from the mother, the calves' behavioural reaction after separation may be alleviated by keeping them satiated after separation (Thomas et al., 2001). Signs of stress at weaning can be reduced when the two events "loss of milk" and "separation from the mother" are separated in time (Haley, 2006; Loberg et al., 2008; Weary et al., 2008). However, others have argued that two-step weaning and fence-line separation prolongs the experienced distress (Enriquez et al., 2010, 2011).

Fence-line separation has not been evaluated for dairy cows and calves before. Artificial selection may have caused both the social behaviour and the maternal innate behaviour of dairy cattle to change (Le Neindre and Sourd, 1984; von Keyserlingk and Weary, 2007). Specifically, a study by Sandem and Braastad (2005) found that cows from a modern dairy breed showed a less strong response to separation compared to cows from a local breed and this response may thus also be different from that of beef cattle. In addition, there is a need for a separation method which accommodates post-separation milk feeding of the calf. This reflects the situation in organic herds where it is required that dairy calves receive whole milk for 12 weeks (EU, 2007), whereas few farmers let the calf suckle this long. We therefore studied fence-line separation while applying a reverse two-step method, meaning that calves were separated from the dams while they continued to receive milk.

The aim of the study was to compare two separation methods, FL with visual and physical contact and separation by a solid wall (SW) allowing auditory contact only. We predicted that FL separation of calves continuing to receive milk would decrease behaviours like vocalizations, pacing and alertness of cows and calves when compared to separation of calves receiving milk but allowed merely auditory contact with the dam.

2. Materials and methods

2.1. Experimental design

This study was carried out on a commercial Norwegian organic dairy farm. Between January 2010 and February 2011, eight batches of two cow–calf pairs were separated as part of the trial. All cow–calf pairs were separated after 8 weeks of free suckling (mean ± SD: 54 ± 11 days). Each batch (containing two calves and two cows) was randomly allocated to one of two separation treatments: Separation with a fence-line (FL, 8 cow–calf pairs) providing auditory, visual and tactile contact or separation with a solid wall (SW, 8 pairs) providing merely auditory contact. All procedures were in accordance with the regulations controlling experiments/procedures in live animals in Norway, and the study complies with the policies relating to animal ethics.

2.2. Animals, feeding and management

All cows and calves were of the Norwegian Red breed (a dual-purpose breed) (GENO, 2012). Median cow parity in the trial was 2.5. The cows were fed silage and hay from a round bale feeder semi ad libitum and new feed was provided twice a day at 07.00 h and 15.00 h. The animals had free access to water. Cows were milked at 06.00 h and 16.00 h in an adjacent milking parlour and they were fed an
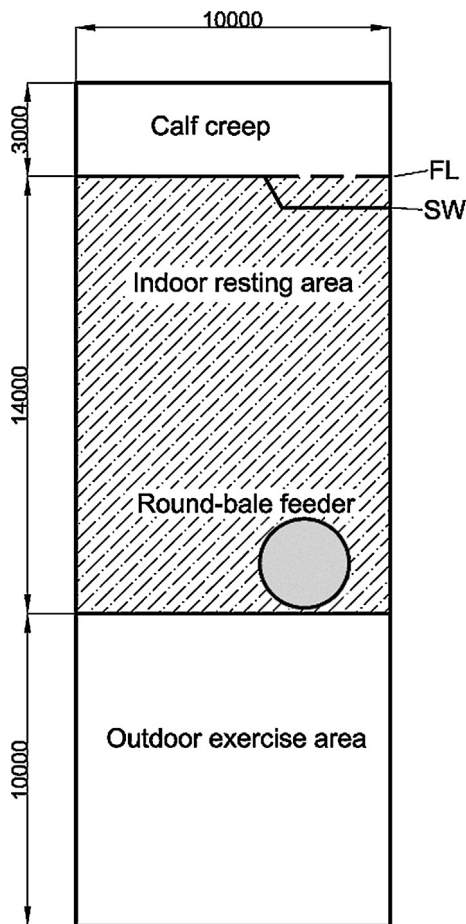
Fig. 1. Outline of the test area containing the calf creep, the indoor resting area and the outdoor exercise area. Measurements are given in millimetres (mm).

individual ration of standard concentrates during milking. The cows had a resting area inside an uninsulated building with deep straw bedding (14 m × 10 m) and free access to an outdoor enclosure (10 m × 10 m).

The cows were moved to an individual calving pen some days prior to parturition. Approximately three days after giving birth, the calf and its dam were allowed to join the milking cow group of 17 cows. During the suckling period, calves suckled the cows freely. A calf creep (10 m × 3 m) with deep straw bedding and with ad libitum access to hay, water and concentrates was located adjacent to the cows' indoor resting area (Fig. 1). Calves could also feed from the cows feeding rack. Only cows with an uncomplicated parturition were included. Calves that were not observed to suckle within 6 h after birth were assisted to do so according to standard farm practice (7 out of 16 calves). Later, all cows nursed their calves without any need of assistance throughout the suckling period. Animal health was monitored daily and there were no signs of disease

2.3. Separation treatments

At time of separation, the gate separating the cow pen from the calf creep (hereafter referred to as calf pen) was

closed at 10.00 h. FL calves (6 bull and 2 heifer calves) were allowed visual and physical contact with the cows over and through a wooden plank fence between the indoor resting area and the calf pen (Figs. 1 and 2). Cows were able to stretch the head over the fence and lick the calf, whereas the calves could put the head through the fence but were not able to suckle. In the SW treatment, the calves (6 heifer and 2 bull calves) were prevented from seeing or physically interacting with their dams as a two metre high, solid barrier was put up between the calf creep and indoor resting area. The cows and calves were, however, able to hear each other. The outline of the solid wall (positioned in front of and parallel to the fence-line) permitted calves to put the head through the inner fence and cows could stretch the nose over the top of the wall.

After separation, the calves were fed fresh, warm, whole milk through hand feeding three times per day (06.00, 14.00 and 16.30 h) with teat bottles. At each feeding, the calves were offered 2 L of milk. In this way, we tried to keep the calves satiated at the time of observation. All calves accepted hand feeding within 24 h and easily got used to it.

2.4. Behavioural observations

Direct observations were carried out on the day of separation (day 0) and for four consecutive days (days 1–4) for two bouts of 2 h between 14.30 and 19.00 h (break from 16.30 to 17.00). Prior to the start of every observation day, the cows and calves were allowed 15 min to habituate to the presence of the observer. Only behaviours indicative of post-separation stress were included in the ethogram: alert behaviour and pacing which were recorded mutually exclusive. In addition we also recorded whether or not the cow or calf stayed close to the separation barrier (Table 1) which was made possible by white tape markings on the walls of the cow pen and the calf creep: 2 m and 1.5 m from the separation barrier respectively. These behaviours were scored using instantaneous sampling every 5 min. All high- and low-pitched vocalizations and head out of pen were recorded mutually exclusive and scored in a continuous manner. Care was taken to reduce potential inter-observer bias by precisely defining behaviours and training the three observers. Blinding of the observers was not possible.

2.5. Statistical analyses

SPSS (v. 21, IBM) was used to calculate median values: For continuously recorded behaviours as frequency of recordings per day (i.e. per 4 h period), and for instantaneously recorded behaviours, as the percentage of total observations of which the behaviour occurred. None of the behavioural parameters were normally distributed, and in order to reduce the number of comparisons, we used the median daily frequency (continuously recorded behaviours) or the median daily percentage (instantaneously recorded behaviours) of the behaviour to calculate
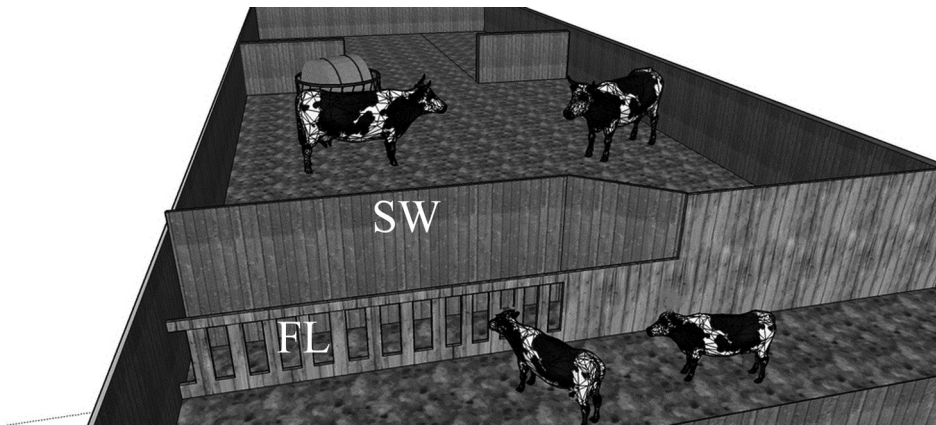
Fig. 2. Diagram showing the setup of the separation treatments. In the solid-wall (SW) treatment the cow–calf pairs were separated with a solid wall (SW) as depicted. In the fence-line (FL) treatment, the solid wall was removed and the cow–calf pairs were separated only by the wooden plank fence.

area under the curve (AUC):

AUC behaviour

$$= \left[\frac{\text{behaviour day 0} + \text{behaviour day 1}}{2} \right] * 1$$

$$+ \left[\frac{\text{behaviour day 1} + \text{behaviour day 2}}{2} \right] * 1$$

$$+ \left[\frac{\text{behaviour day 2} + \text{behaviour day 3}}{2} \right] * 1$$

$$+ \left[\frac{\text{behaviour day 3} + \text{behaviour day 4}}{2} \right] * 1$$

To detect possible differences between the two separation treatments, two samples Wilcoxon test was applied (R gui, vers. 3.0.3). For descriptive purposes, the data from high-pitched and low-pitched vocalizations (frequencies per 4 h) were divided into low, medium and high vocal responses by dividing the range in three with equally sized widths. There was an imbalance in calf sex between the two treatments. To evaluate if the sex imbalance affected the behavioural measures, we conducted additional analyses. Within each treatment, we compared each post-separation behavioural response for heifer calves to that of bull calves. For these analyses we used AUC as described above. We found no significant sex differences in any of the behavioural responses. Therefore, only the joint results for the 8 calves per treatment are presented. All tests are two-tailed, and a significant result was reported at $P < 0.05$.

3. Results

Cows and calves in both treatments were clearly vocal during the first days subsequent to separation and the post-separation response to separation peaked during day 0–2.

3.1. Calf responses

SW calves performed significantly more high-pitched vocalizations ($P = 0.003$, Fig. 3a) and were more often recorded to show alert behaviour ($P = 0.003$, Fig. 3b)

Table 1
Description of behaviours indicative of post-separation stress recorded during direct live observations of each focal cow or calf during the first five days after fence-line separation (FL, $n = 8$ cow–calf pairs) or separation with auditory contact (SW, $n = 8$ cow–calf pairs). Observations were either continuous (frequencies counted per hour), or instantaneous (fixed time point interval sampling recorded every 5 min).

Behaviour	Definition	Recorded for	Method of observation
High-pitched vocalization[a]	Every single open mouthed "muh" vocalization with inhalation between two occurrences	Cow and calf	Continuous
Low-pitched vocalization[a]	Every single closed mouthed 'mm' type vocalization with inhalation between two occurrences	Cow and calf	Continuous
Head out of pen	Cow or calf puts its tip of the nose/head through openings- or over the fence or stands <5 cm with any body part close to fence	Cow and calf	Continuous
Alert behaviour	Standing or lying down in any resting position with high-head posture, pointed ears with focus towards the indoor resting area (calf) or the calf creep (cow)	Cow and calf	Instantaneous
Close to the separation barrier	Animal staying within 2 (cow) or 1.5 (calf) metres of the separation barrier	Cow and calf	Instantaneous
Pacing	Calf is moving forth and back parallel to, within 1 m of the fence	Calf	Instantaneous

[a] Only vocalizations for which the mouth of the cow or calf could be visualized were recorded.
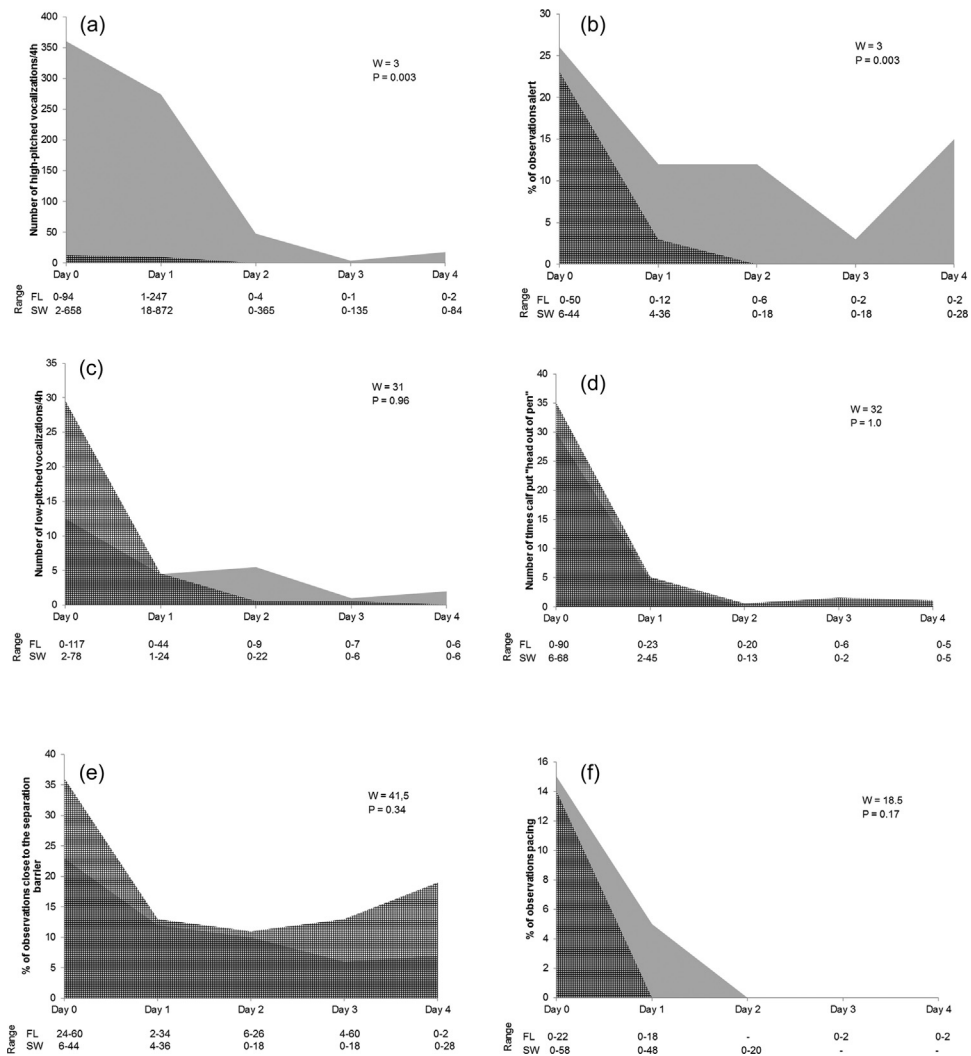
Fig. 3. (a–f) Effect of cow–calf separation through a fence-line allowing physical contact (FL, ⊞ $n = 8$ calves) or a solid wall allowing merely auditory contact (SW, ■ 8 calves) on calf behaviours indicative of stress as recorded during a 4 h period at days 0 (day of separation), 1, 2, 3 and 4. Graphs show, respectively: (a) high-pitched vocalization (median frequency and range), (b) time spent alert (median percentage of observations and range), (c) low-pitched vocalizations, (d) head out of pen, (e) time spent close to the separation barrier, and (f) pacing. Potential differences between the two separation methods were tested for using Wilcoxon two sample test for the total area under the curve. Results are given by W and P-values.

compared to the FL calves. Five of the SW calves were highly vocal (median and range of high-pitched vocalizations per 4 h), both at day 0 (360, 2–658), day 1 (274, 18–872) and day 2 (48, 0–365), whereas the number of high-pitched vocalizations in the FL-treatment was categorized as a low vocal response for all calves even at day 0. Also, time spent alert was higher at day 0 than later, but the behaviour continued to be high for SW calves.

The number of low-pitched vocalizations appeared to be higher in the FL-treatment than in the SW-treatment on day 0 (Fig. 3c), but the total behavioural response over days 0–4 was not significantly different between the treatments. Three of the FL-calves were categorized as highly vocal (79–117 low-pitched vocalizations/4 h). All calves except two (one from each treatment) were observed to put their head through the fence (Fig. 3d) at day 0, and there were large individual differences but no effect of treatment. On

day 0, a few FL calves were seen to perform this behaviour when the dam was standing close to the separation barrier. This behaviour declined rapidly and was rarely seen after day 1. All calves spent more time near the separation barrier at day 0 than later, and there was no treatment effect (Fig. 3e). Pacing was performed by most calves on day 0 (Fig. 3f), by six SW and three FL calves at day 1 and thereafter only sporadically by one FL calf and two SW calves. Treatment had no significant effect on this behaviour.

3.2. Cow responses

The post-separation behaviours (Fig. 4a–e) of cows were not statistically different between FL and SW cows. As with the calves, no FL cows but three SW cows were highly or medium vocal with respect to high-pitched vocalizations (523–783, or 262–522 high-pitched vocalizations/4 h
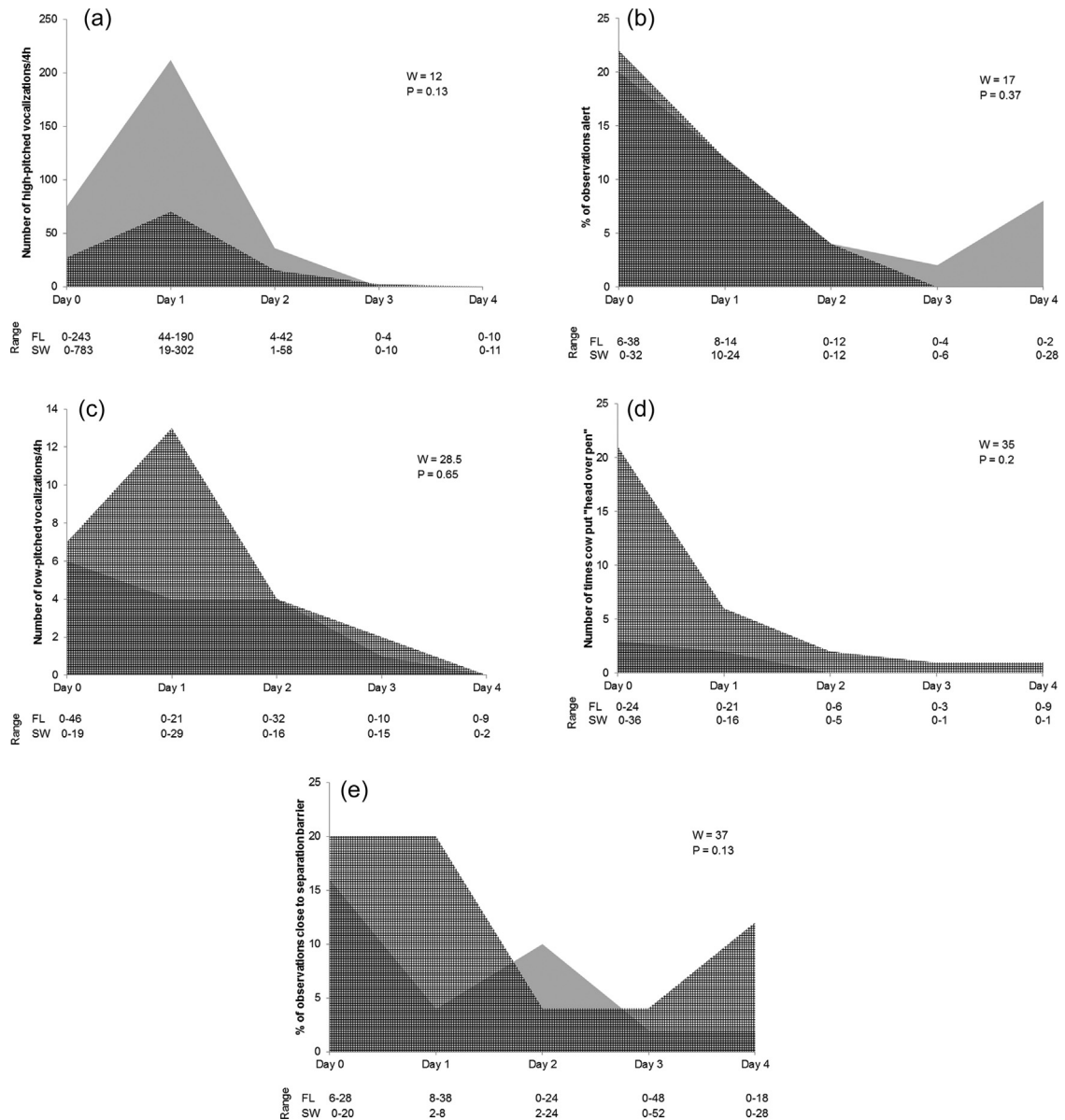
Fig. 4. (a–e) Effect of cow–calf separation through a fence-line allowing physical contact (FL, ⊞ *n* = 7 cows) or a solid wall allowing merely auditory contact (SW, ▪ *n* = 7 cows) on cow behaviours indicative of stress as recorded during a 4 h period for days 0 (day of separation), 1, 2, 3 and 4. Graphs show, respectively: (a) high-pitched vocalization (median frequency and range), (b) time spent alert (median percentage of observations and range, (c) low-pitched vocalizations, (d) head out of pen and (e) time spent close to the separation barrier. Potential differences between the two separation methods were tested for using Wilcoxon two sample test for the total area under the curve. Results are given by *W* and *P*-values.

respectively). With respect to low-pitched vocalizations, two FL cows were highly vocal (31–47 low-pitched vocalizations/4 h) at day 0 and 2. All cows except two, one from each treatment, were observed to put their head out of the pen. All cows spent some time close to the separation barrier, and for both treatments this behaviour varied greatly both individually and from day to day. Alert behaviour was shown by all cows during the first 3 days, and whereas most SW cows continued to show alert behaviour throughout the trial, only a few FL cows were occasionally observed performing this behaviour beyond day 2.

4. Discussion

Our results showed that calves separated with a SW, allowing merely auditory contact with the dams, performed significantly more high-pitched vocalizations, and spent significantly more time in an alert body posture than fence-line separated calves. For cows, no significant differences were found.

Cows and calves in both treatment groups showed a distinct reaction during the first days subsequent to separation, which is in agreement with previous studies (e.g.

Lidfors, 1996; Marchant-Forde et al., 2002; Haley and Stookey, 2005), but in contrast to others (Hopster et al., 1995). The behavioural reaction was most probably caused by the inability to be together as suggested by Loberg et al. (2008). Hunger, or an unfamiliar environment, may also cause similar behavioural reactions (Thomas et al., 2001; Boe and Faerevik, 2003), but the calves in our study were accustomed to the calf creep and recently fed with milk. Our study thereby confirms that separation of the cow and calf is a welfare concern, in agreement with others (Flower and Weary, 2001).

This is the first study to quantitatively assess two different cattle vocalizations in response to forced separation; low-pitched "mm" vocalizations and high-pitched "muhh". The results suggest that high-pitched vocalizations are performed when calves cannot find their dam, as described previously (Kiley, 1972; Haley, 2006; Padilla de la Torre et al., 2015). These vocalizations were more frequently performed by SW calves, and may thus signal an unfulfilled motivation to reunite. Padilla de la Torre et al. (2015) showed that high-pitched vocalizations or "High Frequency Calls" preceded reuniting and nursing when cow–calf pairs kept under free-ranging conditions were in different parts of the field without visual contact. Several authors have found that beef cows and calves vocalize less when separation occurs by a fence-line (Stookey et al., 1997; Price et al., 2003). This was found even when using electric fencing which presumably limits physical contact (Price et al., 2003). Fence-line weaning is also found to decrease the weaning response for farmed wapiti and foals (McCall et al., 1985; Haigh et al., 1997). Interestingly, we often found that a cow and its calf were both "highly vocal" indicating that the vocal response may rub off within a cow–calf pair. In a study where cows were provided no physical, but only visual- and auditory contact to their calf after separation, the cows showed more behavioural reactions (Stěhulová et al., 2008) whereas we found no effect of the separation method on cow behaviour. The increased vocal response and alert behaviour of SW calves in our study may thus provide support for the theory that if fence-line separation is not done into direct adjacent pens, remote and abrupt separation may be better (Haley, 2006).

Calves separated with a solid wall allowing merely auditory contact showed a higher frequency of alert behaviour; high-head posture, pointed ears with focus towards the cow pen. Enriquez et al. (2010) described these behaviours in beef calves as seeking behaviour in response to both fence-line separation and total separation from the dam. The observation that FL calves showed less alert behaviour compared to SW calves, may indicate that seeing and being allowed to physically touch their dams, to some extent satisfied their desire to reunite with the cow.

Studies of natural weaning in zebu cattle (*Bos indicus*), show that the cow prevents her calf from nursing over a 2-week period, but continues to associate with her offspring long after weaning and chooses them as grooming and grazing partners for many years (Reinhardt and Reinhardt, 1981). Fence-line separation contains elements reflecting natural weaning: the cow and calf continue to have some degree of contact although suckling is prevented.

Enriquez et al. (2010) found that the calves attempted numerous unsuccessful suckling attempts through the fence, and thus concluded that fence-line separation may increase frustration. Although we did not record nursing attempts systematically, we did observe a very few such attempts, and only during the first day; i.e. before the calves were accustomed to being handfed. We fed the calves prior to both of the 2 h observation bouts, assuming some level of satiation during the behavioural observation. Still, most of the calves were vocal and SW calves performed more high-pitched vocalizations. Since feeding the calves milk after separation is known to decrease calf vocalizations (Thomas et al., 2001), our results indicate that the calves are motivated to reunite with the dam although at least to some extent nutritionally independent from her as suggested by Newberry and Swanson (2008). As the calves continued to receive milk, the vocalizations may thereby first of all be a signal for maternal care per se. The combination of satiation from milk and physical contact to the dams may explain why the FL calves performed less high-pitched vocalizations. Keeping close to each other represents a natural response of cows and calves at weaning irrespective of whether or not the calf is suckling (Veissier and Le Neindre, 1989).

Most of the recorded behaviours declined in intensity over the observation period, and most of the behavioural response was observed during day 0–2 as reported by others (Enriquez et al., 2010). Also, most of the post-separation behavioural response was unaffected by the level of physical contact between cow and calf. For example, both cows and calves separated by fence-line continued to spend some time near the fence (i.e. <2 m from the fence) and put their heads out of the pen. Also, cows and calves in both treatments performed low-pitched vocalizations. Other studies have shown that by the third day after separation, both cows and calves spend most of their time away from the fence indicating adaptation to separation (Enriquez et al., 2010). Placing the head outside the pen has been interpreted as an attempt of dam and young to become reunited, and has been used as a sign of post-separation stress (Flower and Weary, 2001; Loberg et al., 2007; Stěhulová et al., 2008). Fence-line pacing is thought to reflect an increased activity with the aim to reunite with the cow (Watts, 2001), and was performed by calves in both treatments during the first two days.

The behavioural response at separation should be weighed against the benefits of cow–calf suckling as compared to artificial rearing where the calf is separated from the dam shortly after birth. Suckling calves have improved weight gains (Flower and Weary, 2001; Grondahl et al., 2007), may be healthier (Weary and Chua, 2000) and exhibit behaviours related to positive affective states (Duve et al., 2012; Johnsen et al., 2015). Dairy cows nursing a calf have the ability to express maternal behaviour and positive effects have also been shown with respect to performance (Bar-Peled et al., 1997). As reviewed by Flower and Weary (2003) and Krohn (2001), the positive effects of being together on health and welfare outweigh the stress at separation. At a whole, we conclude that the long suckling period of the calves in our study justifies the 3 days of separation distress for cows and calves. A reverse

two step separation as practiced in this study is a practical way to separate cows and calves while the calf continues to receive milk. Collectively, the calf's welfare during separation largely depends on whether or not this stressful event coincides with that of weaning (Weary et al., 2008).

A limitation of this study is the relatively small number of cow–calf pairs included. Also, by including physiological measures of distress conclusions could potentially have been strengthened. In addition, the two treatment groups were imbalanced with respect to sex. An effect of sex cannot be ruled out although statistical analyses revealed no such differences. Other authors have also found that heifer and bull calves may be equally attached to their dams during the first months of age (Bouissou et al., 2001) and that calf calls are encoded by age but not sex (Padilla de la Torre et al., 2015).

5. Conclusion

Although fence-line separation did not affect cow behaviour, actual physical contact in the days subsequent to separation did reduce high-pitched vocalizations and alert behaviour in the calves. However both cows and calves showed a distinct behavioural response to separation, which encompasses the need for further studies to develop a more optimal pre-weaning separation method. Recordings of high-pitched vocalizations seem to be a promising tool for assessing distress at separation.

Conflict of interest

None declared.

Acknowledgements

References

Bar-Peled, U., Robinzon, B., Maltz, E., Tagari, H., Folman, Y., Bruckental, I., Voet, H., Gacitua, H., Lehrer, A.R., 1997. Increased weight gain and effects on production parameters of holstein heifer halves that were allowed to suckle from birth to six weeks of age. J. Dairy Sci. 80, 2523–2528.

Boe, K.E., Faerevik, G., 2003. Grouping and social preferences in calves, heifers and cows. Appl. Anim. Behav. Sci. 80, 175–190.

Bouissou, M.-F., Boissy, A., Le Neindre, P., Veissier, I., 2001. The social behaviour of cattle. In: Keeling, L.J., Gonyou, H.W. (Eds.), Social Behaviour in Farm Animals. CABI International, Wallingford, UK.

Debio, N., 2005. 2005-10-04: Forskrift om økologisk produksjon og merking av økologiske landbruksprodukter og næringsmidler. www.lovdata.no

Duve, L.R., Weary, D.M., Halekoh, U., Jensen, M.B., 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.

Enriquez, D., Hotzel, M.J., Ungerfeld, R., 2011. Minimising the stress of weaning of beef calves: a review. Acta Vet. Scand., 53.

Enriquez, D.H., Ungerfeld, R., Quintans, G., Guidoni, A.L., Hotzel, M.J., 2010. The effects of alternative weaning methods on behaviour in beef calves. Livest. Sci. 128, 20–27.

EU, 2007. Council Regulation (EC) No. 834/2007 on Organic Production and Labelling of Organic Products and Repealing Regulation (EEC) No. 2092/91 of 28 June 2007.

Flower, F.C., Weary, D.M., 2001. Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth. Appl. Anim. Behav. Sci. 70, 275–284.

Flower, F.C., Weary, D.M., 2003. The effects of early separation on the dairy cow and calf. Anim. Welf. 12, 339–348.

GENO, 2012. www.geno.no

Grondahl, A.M., Skancke, E.M., Mejdell, C.M., Jansen, J.H., 2007. Growth rate, health and welfare in a dairy herd with natural suckling until 6–8 weeks of age: a case report. Acta Vet. Scand., 49.

Haigh, J.C., Stookey, J.M., Bowman, P., Waltz, C., 1997. A comparison of weaning techniques in farmed wapiti (Cervus elaphus). Anim. Welf. 6, 255–264.

Haley, D.B., 2006. The Behavioural Response of Cattle (Bos taurus) to Artificial Weaning in Two Stages. Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon.

Haley, D.B., Stookey, J.M., 2005. Weaning cattle in two stages reduces the behavior changes typically associated with weaning stress. J. Dairy Sci. 88, 349.

Hopster, H., Oconnell, J.M., Blokhuis, H.J., 1995. Acute effects of cow–calf separation on heart-rate, plasma cortisol and behavior in mutliparous dairy-cows. Appl. Anim. Behav. Sci. 44, 1–8.

Johnsen, J.F., de Passille, A.M., Mejdell, C.M., Bøe, K.E., Grøndahl, A.M., Beaver, A., Rushen, J., Weary, D.M., 2015. The effect of nursing on the cow–calf bond. Appl. Anim. Behav. Sci. 163, 50–57.

Kiley, M., 1972. The vocalization of ungulates. Z. Tierpsychol. 31, 171–222.

KRAV, S., 2012. Regler for KRAV-certifierad produktion. In: KRAV (Ed.), Utgåva 2012. www.krav.se

Krohn, C.C., 2001. Effects of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioural aspects in high producing dairy cows – a review. Appl. Anim. Behav. Sci. 72, 271–280.

Le Neindre, P., Sourd, C., 1984. Influence of rearing conditions on subsequent social behaviour of Friesian and Salers heifers from birth to six months of age. Appl. Anim. Behav. Sci. 12, 43–52.

Lidfors, L.M., 1996. Behavioural effects of separating the dairy calf immediately or 4 days post-partum. Appl. Anim. Behav. Sci. 49, 269–283.

Loberg, J.M., Hernandez, C.E., Thierfelder, T., Jensen, M.B., Berg, C., Lidfors, L., 2007. Reaction of foster cows to prevention of suckling from and separation from four calves simultaneously or in two steps. J. Anim. Sci. 85, 1522–1529.

Loberg, J.M., Hernandez, C.E., Thierfelder, T., Jensen, M.B., Berg, C., Lidfors, L., 2008. Weaning and separation in two steps – a way to decrease stress in dairy calves suckled by foster cows. Appl. Anim. Behav. Sci. 111, 222–234.

Marchant-Forde, J.N., Marchant-Forde, R.M., Weary, D.M., 2002. Responses of dairy cows and calves to each other's vocalisations after early separation. Appl. Anim. Behav. Sci. 78, 19–28.

McCall, C.A., Potter, G.D., Kreider, J.L., 1985. Locomotor, vocal and other behavioural responses to varying methods of weaning foals. Appl. Anim. Behav. Sci. 14, 27–35.

Newberry, R.C., Swanson, J.C., 2008. Implications of breaking mother–young social bonds. Appl. Anim. Behav. Sci. 110, 3–23.

Nicol, A.M., 1977. Beef cattle weaning methods. N. Z. J. Agric. 134, 17–18.

Padilla de la Torre, M., Briefer, E.F., Reader, T., McElligott, A.G., 2015. Acoustic analysis of cattle (Bos taurus) mother–offspring contact calls from a source-filter theory perspective. Appl. Anim. Behav. Sci. 163, 58–68.

Panksepp, J. (Ed.), 1998. Affective Neuroscience: The Foundations of Human and Animal Emotions. Oxford University Press, New York.

Price, E.O., Harris, J.E., Borgwardt, R.E., Sween, M.L., Connor, J.M., 2003. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate. J. Anim. Sci. 81, 116–121.

Reinhardt, V., Reinhardt, A., 1981. Natural sucking performance and age of weaning in zebu cattle (Bos Indicus). J. Agric. Sci. 96, 309–312.

Sandem, A.I., Braastad, B.O., 2005. Effects of cow–calf separation on visible eye white and behaviour in dairy cows – a brief report. Appl. Anim. Behav. Sci. 95, 233–239.

Solano, J., Orihuela, A., Galina, C.S., Aguirre, V., 2007. A note on behavioral responses to brief cow–calf separation and reunion in cattle (Bos indicus). J. Vet. Behav. Clin. Appl. Res. 2, 10–14.

Stěhulová, I., Lidfors, L., Špinka, M., 2008. Response of dairy cows and calves to early separation: Effect of calf age and visual and auditory contact after separation. Appl. Anim. Behav. Sci. 110, 144–165.

Stookey, J.M., Schwartzkopf-Genswein, K.S., Waltz, C.S., Watts, J.M., 1997. Effects of remote and contact weaning on behaviour and weight gain of beef calves. J. Anim. Sci. 75 (Suppl. 1), 83.

Thomas, T.J., Weary, D.M., Appleby, M.C., 2001. Newborn and 5-week-old calves vocalize in response to milk deprivation. Appl. Anim. Behav. Sci. 74, 165–173.

Veissier, I., Le Neindre, P., 1989. Weaning in calves: its effects on social organization. Appl. Anim. Behav. Sci. 24, 43–54.

Ventura, B.A., von Keyserlingk, M.A.G., Schuppli, C.A., Weary, D.M., 2013. Views on contentious practices in dairy farming: the case of early cow–calf separation. J. Dairy Sci. 96, 6105–6116.

Vidensenteret for økologisk landbrug, 2012. Oversikt over regler for økologisk landbruk, Danmark. www.okolandmand.nu

von Keyserlingk, M.A.G., Weary, D.M., 2007. Maternal behavior in cattle. Horm. Behav. 52, 106–113.

Watts, J.M., 2001. Vocal Behaviour and Welfare in Cattle. Univ. of Saskatchewan, Saskatoon, Canada.

Weary, D.M., Chua, B., 2000. Effects of early separation on the dairy cow and calf. 1. Separation at 6 h, 1 day and 4 days after birth. Appl. Anim. Behav. Sci. 69, 177–188.

Weary, D.M., Jasper, J., Hotzel, M.J., 2008. Understanding weaning distress. Appl. Anim. Behav. Sci. 110, 24–41.

1

2

3 **The effect of large milk meals on digestive physiology and behaviour in dairy calves**

4

5

6 **Kristian Ellingsen*[1], Cecilie M. Mejdell*, Nina Ottesen†, Stig Larsen† and Ann**

7 **Margaret Grøndahl†**

8

9 *Norwegian Veterinary Institute, PO Box 750 Sentrum, N-0106 Oslo, Norway

10 †Norwegian University of Life Sciences, Faculty of Veterinary Medicine and Biosciences,

11 PO Box 8146 Dep, N-0033 Oslo, Norway

12

13 [1]Corresponding author:

14 Kristian Ellingsen

15 Norwegian Veterinary Institute

16 PO Box 750 Sentrum

17 N-0106 Oslo

18 Norway

19 Kristian.Ellingsen@vetinst.no

20 Phone: +47 23 21 63 77

21 Fax: +47 23 21 60 01

22

23

24   Abstract

25   It is commonly believed that young calves should not be fed more than about two liters of

26   milk per meal. If calves are fed beyond this volume, it is said that the capacity of the

27   abomasum may be exceeded and that milk could enter the rumen. This can disturb the

28   microbial flora/fauna of the rumen and enhance the risk of indigestion, diarrhoea and reduced

29   growth. The aim of this study was to examine the effect of large milk meals on digestive

30   physiology and behaviour in dairy calves. Six calves (19-23 days of age at the beginning of

31   the experiment) were fed two litres of warm whole milk by teat bottle three times per day,

32   which was the recommended Norwegian feeding regime at the time. The calves were given

33   free access to hay, concentrates and water. During three morning feeding sessions, each

34   separated by 48 hours, all calves were offered larger meals. The offered amounts were

35   calculated according to the within patient 3-level Response Surface Pathway (RSP) design.

36   The milk given on the three test days contained a contrast medium (barium sulphate

37   ($BaSO_4$)), and the animals were radiographed before, during and immediately after intake to

38   reveal whether milk entered the rumen. Four out of the six calves drank more than five litres

39   in one meal and the highest voluntary intake was 6.8 liters in one meal (13.2% of BW).

40   Abdominal radiographs showed that the abomasum has great capacity for expansion. Milk in

41   the rumen was not observed in any of the calves, regardless of intake. The behaviour of the

42   calves was observed for two hours after each test session. No behavioural signs of abdominal

43   pain or discomfort were observed regardless of intake. The results indicate that when warm

44   whole milk is administered from a teat bottle, farmers can increase the amount of milk they

45   offer their calves beyond 2-3 litres per meal without any risk of milk entering the rumen.

46   Hence, farmers who want to feed their calves more milk can do so by increasing meal sizes,

47   and not necessarily by introducing an additional meal.

48

49   Key words: milk meal size, abomasal capacity, backflow, ingestion, indigestion

1. Introduction

1.1. Stomach development

During the first weeks of life, calves are functionally monogastric and milk is the primary source of nutrition. Upon drinking milk, the oesophageal (reticular) groove (*sulcus retículi)* is activated and the milk is shunted directly past the forestomachs to the abomasum [1]. A number of factors trigger this oesophageal reflex, including sucking behaviour, warm milk, the position of the calf's head while drinking [1] and familiarity with the feeding method [2].

For newborn and very young calves, milk in the forestomachs is usually unproblematic. The rumen, along with the reticulum and omasum, is not yet developed and empties into the abomasum within hours [3]. For calves with ruminal development (2-3 weeks and older) [4], large quantities of milk in the rumen may pose a problem. The lactose is converted to lactic acid or other organic acids, or the milk protein may rot, which again may lead to a change in pH, subsequently affecting the rumen microflora causing indigestion, diarrhoea and reduced growth [1].

Milk is thought to enter the rumen in one of two ways. The first is through insufficient closure of the oesophageal groove [5]. The second results from overfeeding fluids beyond the capacity of the abomasum, causing backflow into the reticulorumen [5-7]. While the scientific origin remains unclear, it is widely believed that the capacity of the abomasum is about 2 litres, and that milk meal sizes beyond this volume will cause milk to enter the rumen. In a study from 2012, Flor and Sanftleben also calculated the abomasal volume to be less than 2 litres based on computed tomography (CT) scans [8]. They did not, however, report the size of the offered milk meal or actual intake by the calf.

3

1.2.   Milk Feeding in Dairy Calves

Traditionally, dairy calf milk feeding systems have been based on daily feeding rates of 8–10% of body weight (BW) [9]. This feeding regime is highly restrictive compared to ad libitum feeding. Research has shown that calves allowed ad libitum access to milk from artificial teats, drink 8-10 litres [10]. Calves allowed to suckle drink up to 12-15 litres of milk per day at 2-4 weeks of age [11, 12, Grøndahl, unpublished]. Khan et al. [13] recommend to feed dairy calves the equivalent of 20% of BW per day based of a comprehensive review article. This recommendation is more in line with the natural milk intake level of dairy calves. The Norwegian milk feeding recommendations for dairy calves were also recently increased from 6 to 8 litres per day [14].

Until the calf is 3-4 weeks old, restrictive milk feeding can result in the calves being unable to meet their daily energy requirements [15], leading to chronic hunger [16]. One way of feeding the calf more milk is to introduce an additional meal. This increases the workload and is therefore often undesirable in farms without automated milk feeders. Another way is to increase the meal size. Fear of exceeding the abomasal capacity causing milk to enter the rumen, however, is a major reason for restrictive meal size in dairy calves.

1.3.   Aim

The aim of this study was twofold. First, we aimed to use radiographic studies to examine the effect of voluntary intake of large milk meals on digestive physiology in dairy calves. In other words, we wanted to determine how much milk a dairy calf can drink from a teat bottle in one meal before the abomasal capacity is exceeded and milk enters the rumen. Second, we wanted to investigate potential behavioural indicators of abdominal pain or discomfort resulting from intake of large milk meals and milk entering the rumen.

4

2. Material and methods

2.1. Pilot studies

Using a 15 days old heifer calf, two pilot studies were conducted. First, a milk:barium sulphate ($BaSO_4$) (Bracco, MIXOBAR® COLON (1g/ml)) ratio of 6:1 was found to give satisfactory contrast and was readily drunk by the calf. Second, radiography was carried out on the standing calf to assure that milk in the rumen could be distinguished from milk in the abomasum, as shown by Lateur-Rowet and Breukink [3] in calves and Phillipson [17] in lambs. One decilitre barium sulphate solution was administered to the rumen through an oesophageal tube (Figure 1A). Immediately after, the calf was allowed free intake of warm whole milk mixed with the contrast agent through a small aperture teat, in accordance with the test calves. The radiographs show how the abomasum and rumen can easily be discerned after intake of four litres of milk (Figures 1B).
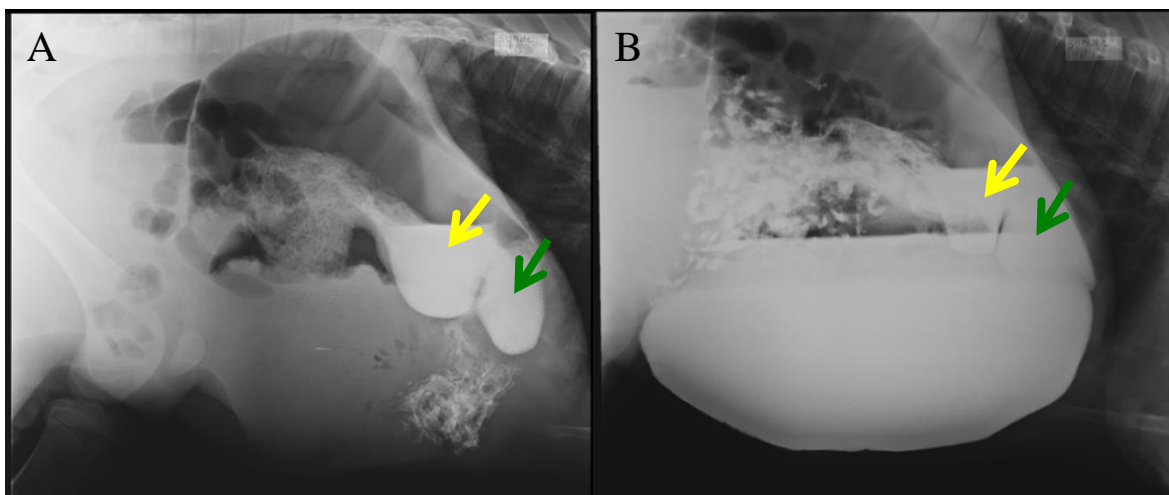
Figure 1. Lateral cranial abdominal radiography of the calf (head oriented to the right) after administration of 100 ml barium sulphate ($BaSO_4$) using an oesophageal tube (A) and after consuming 4 litres of milk mixed with contrast solution ($BaSO_4$) (B). Note that rumen (yellow arrow) and reticulum (green arrow) can be differentiated from the abomasum. Some milk mixed with contrast has been passed to small intestine. (2-column fitting image).

120   2.2.   The test animals

121   Seven Norwegian Red calves (four heifers and three bulls) born only four days apart were

122   borrowed from a dairy farm south of Oslo, Norway. The animals were 19-23 days of age at

123   the beginning of the experiment, and weighed between 39.5 and 48.5 kilograms at arrival. The

124   calves were housed in a group pen (4 by 4 meters) at the Norwegian University of Life

125   Sciences. The calves were fed according to the recommended Norwegian feeding regime at

126   the time and received two litres of warm whole milk three times per day at 8 am, 1 pm and 6

127   pm. The animals had free access to water, hay and concentrates. All test animals were

128   weighed on arrival (day 0) and before each X-ray session (Level 1, 2 and 3).

129

130   One of the heifer calves got sick before the test period and was therefore excluded from all

131   data analyses. The calves were cared for according to the national animal welfare legislation

132   and all calves were returned to their owner after the experiment.

133

134   2.3.   The test procedure

135   On test days 3, 5 and 7 (hereafter referred to as Level 1, 2 and 3, respectively), the calves'

136   morning meal was replaced by milk containing the contrast agent. Abdominal radiographs of

137   the standing calves were performed before, during and immediately after completed

138   administration of the milk and barium sulphate mixture (Table 1).

139

140

141

142

143

144

145

146

147 Table 1. A day-by-day schedule for the full duration of the experiment.

148

Age of calves (days)	Day at test facility	Feeding regime (litres of milk)			Action
		8 am	1 pm	6 pm	
0	-				Calving
19-23	0	-	2	2	Transportation from farm
20-24	1	2	2	2	Habituation of new housing
21-25	2	2	2	2	Habituation of new housing
22-26	3	Level 1	2	2	X-ray after morning meal + observation
23-27	4	2	2	2	-
24-28	5	Level 2	2	2	X-ray after morning meal + observation
25-29	6	2	2	2	-
26-30	7	Level 3	-	-	X-ray after morning meal + observation

149

150 Lateral-lateral abdominal computed radiography (Kodak DirectView CR 850) with a focus

151 film distance (FFD) of 140 cm using a grid (potter bucky) on standing calves was performed.

152 Exposure factors were 90 kV and 32 mAs.

153

154 Milk is easily digestible and abomasal emptying in young calves normally starts within a few

155 minutes [18]. We therefore decided to perform the radiographs during and immediately after

156 the milk intake, as this is the time when backflow is most likely to occur. Ruminal emptying

157 can take up to 48 hours [3]. To avoid confusion from barium sulphate potentially remaining in

158 the forestomachs, feeding trials in the current study were performed with 48 hour intervals.

159

160 Teat feeding, and the use of a small aperture teat, are widely recommended in the literature

161 [19-22], and was also the method of choice in this experiment. Compared to non-sucking

162 methods, teat feeding has advantages like stimulating the oesophageal reflex, and causing

163 fewer sequential openings and closings of the oesophageal groove [23]. The small aperture

164 teat used in the experiment allowed a drinking speed of approximately 1.5 litre/minute. To

165 standardize the rate of intake, the same rubber teat was used for all test sequences and all

166 calves.

167   On each of the three test days, continuous live behavioural observations were carried out in

168   situ for two hours after ingestion of the meal to reveal signs of abdominal pain or discomfort

169   according to the ethogram below (Table 2). The ethogram is based on the work by Bourne

170   [24]. All behavioural observations were carried out by the same observer (1st author,

171   ethologist and researcher with experience from behavioural observations and pain assessment

172   in animals). Observations were made for each calf individually and were recorded on a data

173   logging sheet. The observer was not blinded to the performance of the calves during trials due

174   to lack of personnel.

175

176   Table 2. Ethogram used to detect signs of abdominal pain or discomfort. All calves were observed live for two

177   hours after intake of high amounts of milk and observations were made for each calf individually.

Behaviour	Description	Registration
General impression every 30 min	Normal or dull, passive, unresponsive and uninterested in surroundings	Normal (N) or dull (D)
Vocalization/bellowing	High-pitched vocalization	Number of vocalizations per animal
Licking at the abdomen	The animal turns its head and licks at the abdomen	Number of licks per animal
Biting at the abdomen	The animal turns its head and bites at the abdomen	Number of bites per animal
Kicking at the abdomen	The animals kicks at the abdomen	Number of kicks per animal
Getting up/lying down	The animal partly or fully stands up or lies down	The number of times calf partly of fully stood up or laid down
Rapid, shallow breathing	The animal shows a bout of rapid, shallow breathing	Number of bouts and duration per bout (sec)
Bruxism	The animal shows a bout of grinding teeth	Number of bouts and duration per bout (sec)
Hunched stance	The animal shows a bout of standing with its head low and back arched	Number of bouts and duration per bout (sec)

178

179   In addition to observing behaviours indicative of abdominal discomfort or pain, diarrhoea

180   (defined as faeces with liquid consistency) was recorded for each calf individually.

181

182   2.4.   Response Surface Pathway (RSP) design

183   The study was performed as an open, non-randomized and single centre trial with a within

184   patient 3-level RSP design [25]. The design consists of three dose levels, in which all the

185   calves will be started on the same dose (m) at the first design level, but thereafter participate

8

186   in the study based only on own obtained results (Figure 2). The dose for the next level is

187   dependent on the initial dose (m) and the dose adjustment factor k and given by the formula

188    $m_i = m_{i-1} \pm \dfrac{m}{k^{i-1}}$. The upper dose level (D_U) is expressed by a finite geometrical series. In this

189   study with three design levels gives $D_U = \dfrac{m(k^n - 1)}{(k^n - k^{n-1})}$. With a starting dose of m=4 litre and an

190   upper dose of D_U=7.0 litre, gives a k-adjustment factor of k=2.0.

191

192   The grades of negative reactions (milk detected in the rumen or failure to ingest the offered

193   amount) determine the dose for the subsequent levels. If all milk is drunk and nothing enters

194   the rumen, the highest dose is chosen for the next level. If some milk remains uningested

195   (<33%) or traces of milk is seen in the rumen, the second highest dose is chosen for the next

196   level. If a significant amount of milk remains uningested (>33%) or a substantial amount is

197   evident in the rumen, the second lowest dose is chosen, and if the calf does not drink any milk

198   or if all the milk enters the rumen, the lowest dose is chosen for the next level.

199

200
201 Level 1
202
203
204

Milk in rumen or failure to ingest

Level 2

Milk in rumen or failure to ingest

Level 3

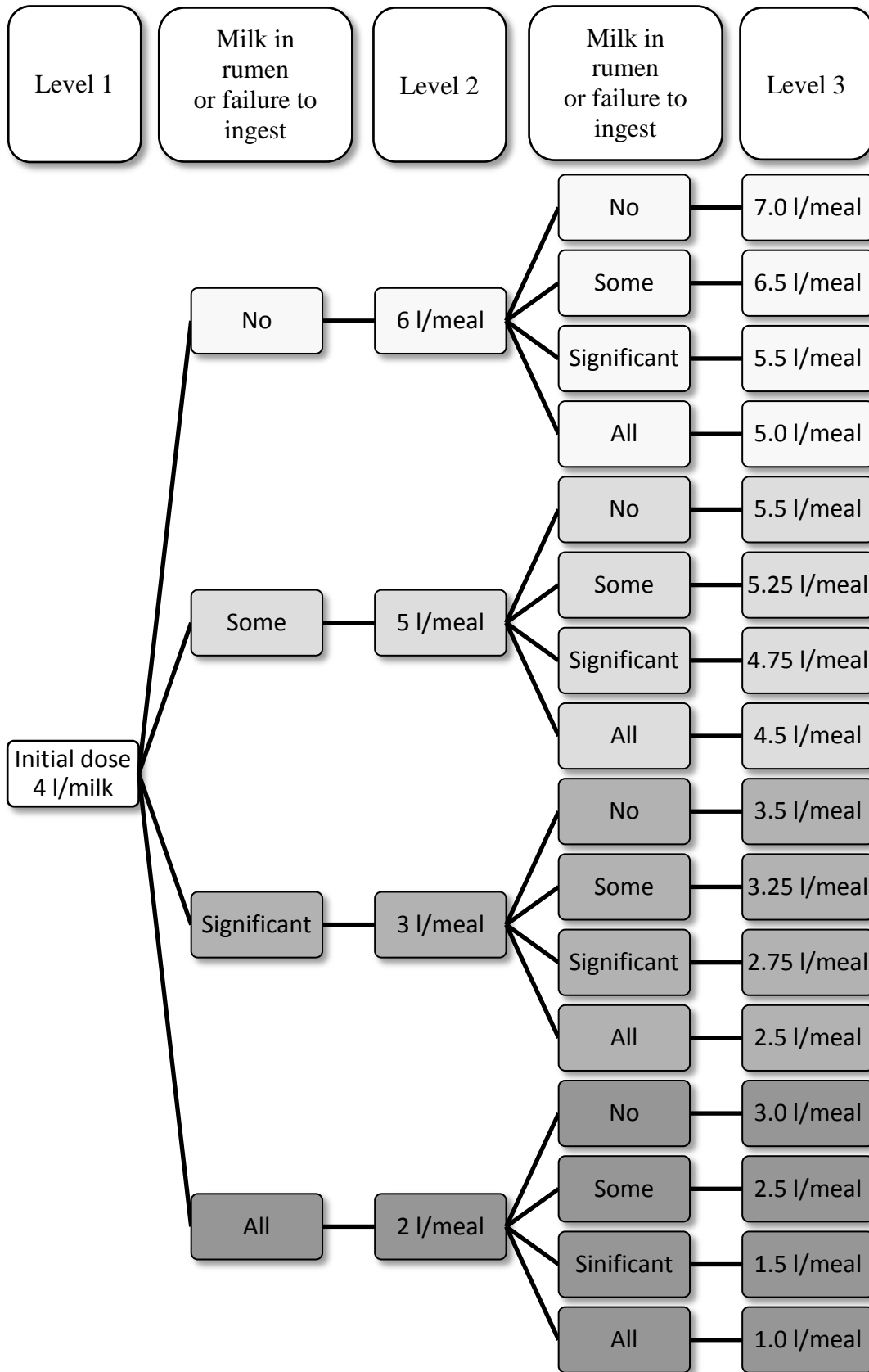|				
	No	6 l/meal	No	7.0 l/meal
			Some	6.5 l/meal
			Significant	5.5 l/meal
			All	5.0 l/meal
	Some	5 l/meal	No	5.5 l/meal
			Some	5.25 l/meal
			Significant	4.75 l/meal
			All	4.5 l/meal
Initial dose 4 l/milk	Significant	3 l/meal	No	3.5 l/meal
			Some	3.25 l/meal
			Significant	2.75 l/meal
			All	2.5 l/meal
	All	2 l/meal	No	3.0 l/meal
			Some	2.5 l/meal
			Sinificant	1.5 l/meal
			All	1.0 l/meal

Figure 2. Response Surface Pathway (RSP) design. RSP with categorized adverse events and dose in litres milk/meal to determine the maximum amount of milk a Norwegian Red calf can drink in one meal without causing milk to enter the rumen. (1.5-column fitting image).

229   2.5.   Statistical Analysis

230   Assumed continuously distributed variables are expressed by mean values with 95%

231   confidence interval constructed by the Student procedure [26]. Categorized or discontinuously

232   distributed variable are expressed in contingency tables [27].

233

234   All comparisons are performed two tailed and differences considered significant for p-values

235   below the level of 5%. Comparisons of continuously distributed variables between design

236   levels are performed by using Analysis of Variance (ANOVA) [26]. Additionally, isotonic

237   regression for multinomial outcome is the suggested model for analysing the material [25,

238   28].

239

240   3. Results

241   All calves drank volumes far exceeding their normal meal size. Milk in the rumen was not

242   detected, regardless of intake. Four of the six calves drank more than five litres in one meal,

243   while two of them drank more than six litres when the highest amounts were offered (Table

244   3). Mean intake from the teat bottle in one meal was 3.8 (8.1% of BW), 4.9 (10.2% of BW)

245   and 5.4 (10.8% of BW) litres of milk on Level 1, 2 and 3, respectively. The highest voluntary

246   intake recorded in the study was 6.8 litres (13.2% of BW).

247

248   On Level1, five of the six calves drank all of the 4 litres they were offered. One calf only

249   drank 2.6 litres (65% of offered amount). This particular calf had a coughing spell during the

250   meal and thereafter lost interest. The low intake on Level 1 had consequences for the amount

251   of milk offered on Levels 2 and 3, at which levels the calf drank all milk offered.

252

253

254   Table 3. An overview of individual intake, mean (SD) and 95% CI in litres and per cent of body weight for the

255   six calves in the experiment. The results are given separately for each of the three Response Surface Pathway

256   levels

Animal	Litres of milk drunk/offered			Intake in per cent of body weight		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
1	2.6/4.0	3.0/3.0	3.5/3.5	5.7	6.3	7.2
2	4.0/4.0	5.0/6.0	5.3/6.5	10.3	12.3	12.8
3	4.0/4.0	5.3/6.0	5.5/6.5	8.5	10.7	10.7
4	4.0/4.0	6.0/6.0	6.8/7.0	7.8	11.9	13.2
5	4.0/4.0	4.8/6.0	4.7/5.5	8.2	9.5	9.0
6	4.0/4.0	5.5/6.0	6.4/6.5	7.8	10.7	12.0
Mean intake (SD)	3.8 (0.6)	4.9 (1.0)	5.4 (1.2)	8.1 (1.5)	10.2 (2.2)	10.8 (2.4)
95% CI	3.2 – 4.4	3.8 – 6.0	4.1 – 6.6	6.5 – 9.6	8.0 – 12.5	8.3 – 13.3

257

258

259   Radiographs of the cranial abdomen of a standing calf show the abomasum before (Figure

260   3A), and after ingestion of 2 litres (Figure 3B), 4 litres (Figure 3C) and 6 litres (Figure 3D) of

261   milk mixed with contrast solution, respectively.

262

263

12

264
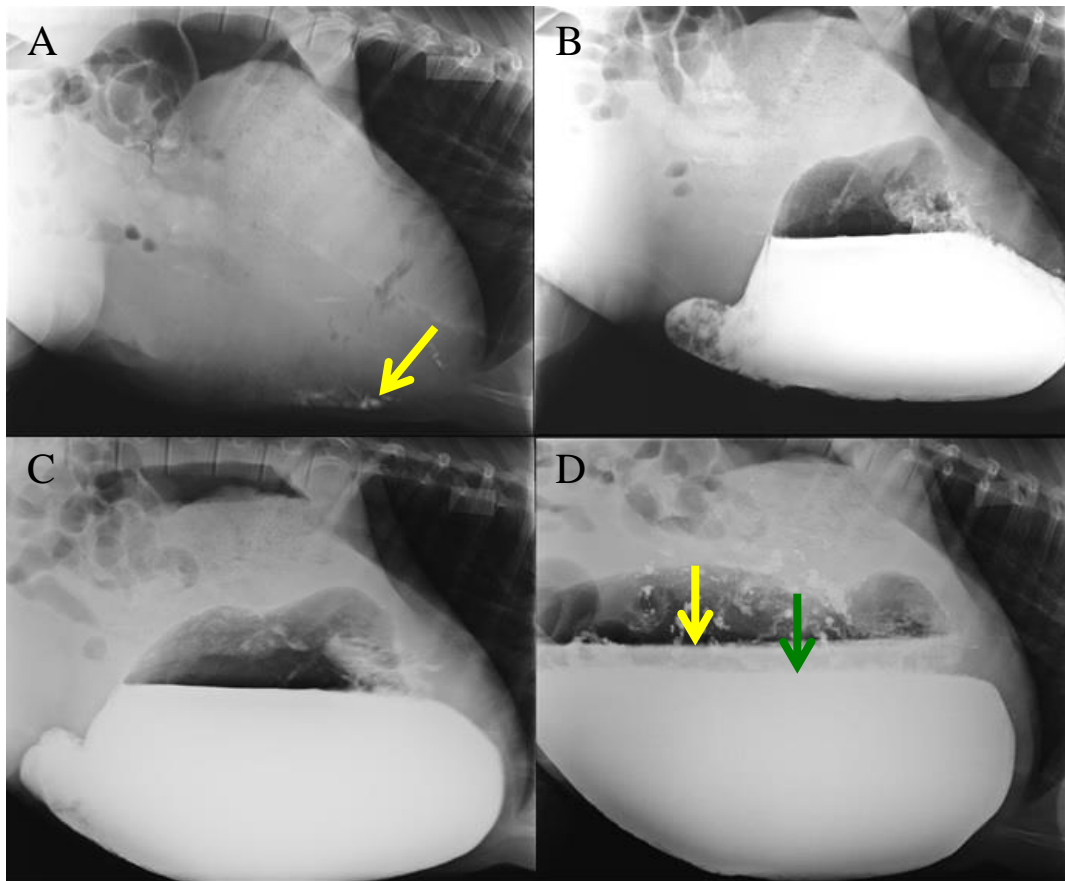
Figure 3. Cranial abdominal radiograph (head oriented to the right) taken at Level 3 before administration (A) and after sucking 2 (B), 4 (C) and 6 (D) litres of milk containing $BaSo_4$ at 6:1 ratio. A: Note a small amount of residual barium sulphate evident in cranioventral aspect of the abdomen consistent with the location of abomasum (arrow). B/C/D: Note that radiopaque material is present in the abomasum, the evident gas bell in the dorsal aspect of abomasum and no visualization of the rumen. D: The lateral abdominal radiographs of standing calves are acquired using horizontal beam. The fluid level refers to an interface between fluid and gas (gas-fluid interface, yellow arrow) in the stomach. In addition to the gas-fluid interface there are occasionally visible fluid-fluid interfaces (green arrow) most likely due to radiographs taken immediately after the calves stopped drinking, before the fluid finds its true level, causing layering of the fluid. (2-column fitting image).

With regards to the behavioural indicators of abdominal pain or discomfort, a low frequency of licking at the abdomen (performed by 2 calves) and lying down/getting up (performed by all calves) were observed among the animals. The highest number of lying down/getting up behaviours performed by one calf was five times within one observation period. No diarrhoea was observed in any of the test animals. Table 4 shows the accumulated observations for all

13

280   six calves per level. The numbers in brackets show the number of animals that carried out the

281   behaviour.

282

283   Table 4. The accumulated occurrence and/or frequency of behaviours related to abdominal pain in calves. The

284   numbers in brackets show the number of animals that carried out the behaviour.

Behaviour	Level 1	Level 2	Level 3
General appearance	N	N	N
Vocalization/bellowing	0	0	0
Licking at the abdomen	3 (1 calf)	4 (2 calves)	0
Biting at the abdomen	0	0	0
Kicking at the abdomen	0	0	0
Getting up/lying down	14 (6 calves)	10 (5 calves)	19 (5 calves)
Rapid, shallow breathing	0 bouts	0 bouts	0 bouts
Bruxism	0 bouts	0 bouts	0 bouts
Hunched stance	0 bouts	0 bouts	0 bouts

285

286

4. Discussion

287

288   The abomasum has a capacity to expand far exceeding what was previously believed.

289   Voluntary intake of up to 6.8 litres of warm whole milk in a 3 week old calf did not cause

290   milk to enter the rumen and no signs of abdominal pain were observed.

291

292   4.1.   Methodological Issues

293   The RSP method was chosen because it effectively narrows down the dose window to

294   pinpoint an exact dosage [25, 28]. The method permits the dose level to be adjusted based on

295   the amount of milk in the rumen, hence allowing us to determine the volume at which the

296   abomasal capacity would be exceeded. Between- and within-patient RSP designs are

297   generally analysed with isotonic regression analysis [25, 28]. However, no meal size in the

298   current study turned out to cause milk to enter the rumen. This means that there are no

299   quantiles to estimate and hence isotonic regression could not be applied to determine an

300   optimal dose level based on abomasal capacity.

301

302   4.2.   Optimal milk feeding

303   The finding that voluntary intake of 5-6 litres of milk in a meal did not cause milk to enter the

304   rumen, does not mean that we can recommend feeding milk meals of that size to dairy calves

305   on a daily basis. The test meals were offered as single meals on three separate occasions, and

306   we did not test how much they would have drunk if offered this amount three times a day.

307   Neither were the long-term effects of high amounts of milk investigated. However, it has, ,

308   been found elsewhere that feeding milk ad libitum [9] or at high levels [29] resulted in higher

309   growth rates without compromising health or reducing solid feed intake after weaning.

310   Appleby et al. also concluded that feeding dairy calves ad libitum from teats allowed them to

311   regulate their own intake while improving performance compared to traditionally fed calves

312   [10]. Our results indicate that farmers who want to feed their calves more milk per day can do

313   so by increasing milk meal size beyond 2-3 litres, instead of introducing an additional meal.

314

315   It could be argued that the knowledge gained in this study indicates that calves can be fed one

316   single, large meal per day rather than several smaller ones. This was not tested, but is

317   probably not recommendable. Large, infrequent meals have been found to have negative

318   effects on calf metabolism and decrease insulin sensitivity [30]. There are also several studies

319   reporting positive effects on weight gain and calf health and welfare as a result of an

320   increased number of milk meals [31-33].

321

322   4.3.   High milk intake and pain behaviour

323   Behavioural observations were included to pick up any signs of abdominal pain or discomfort

324   resulting from the large milk meal. Despite the fact that the calves on several occasions drank

325   milk meals exceeding 10% of BW, no behavioural indications of abdominal pain were

326   observed . Some calves were observed licking at their abdomen. The behaviour was carried

327   out as part of the grooming and was not directed exclusively at the abdomen. Most calves

328   would lie down and get up again during the 2 hour observation periods, but not in a rapid

329   sequence as seen in colic behaviour [24]. On several occasions, the calves raised to eat hay,

330   drink water or as a response to an external stimuli like people looking into the pen. The lack

331   of behaviours indicating pain implies that although high amounts of milk were ingested, the

332   calves are able to control intake in such a way that pain is avoided.

333

334   No diarrhoea was observed in any of the test animals. However, as barium sulphate is an

335   antidiarrhohoeal agent, the contrast solution could have hidden any laxative effects of

336   increased amounts of milk, thus making it hard to draw conclusions from the current study.

337   Khan et al. suggest that a high incidence of diarrhoea is likely to be a problem more related to

338   poor hygiene, management, housing conditions, ventilation and colostrum intake than to the

339   amount of milk [13]. It is also important to note that a calf that drinks high amounts of milk

340   will have more liquid faeces, which is different from pathological diarrhoea.

341

342   4.4.   Future studies

343   The current study was carried out with warm whole milk drunk through a small aperture teat.

344   This means that a number of variables were not investigated. For instance, it is currently

345   unknown whether feeding high volumes of milk replacer would yield the same results. Teat

346   feeding, and the use of a small aperture teat, are widely recommended in the literature Future

347   studies will therefore be aimed at testing if higher volumes of milk can be problematic if

348   managed poorly, e.g. if administered from a teat with a bigger opening or if given straight

349   from an open bucket.

350

351   5. Conclusion

352   Voluntary intake of milk ranging from 3.5 to 6.8 litres in one meal did not cause milk to enter

353   the rumen, and behavioural indicators of abdominal pain or discomfort were not observed

354 regardless of intake. These results indicate that, if warm whole milk is administered by a

355 small aperture teat, farmers may increase the amount of milk they feed their calves per meal.

356

357 Conflict of interest statement

358 The authors declare that there is no conflict of interest associated with this manuscript.

359

360 Acknowledgements

369

370 References

371 [1] Sjaastad, Ø. V., Hove, K., Sand, O. Physiology of domestic animals. 2nd ed. Oslo:

372   Scandinavian Veterinary Press; 2010.

373 [2] Abe, M., Kondoh, K., Shibui, H. Effects of nipple or bucket feeding of milk-substitutes on

374   rumen by-pass and on rate of passage in calves. Br. J. Nutr. 1979,41:175-81.

375 [3] Lateur-Rowet, H. J., Breukink, H. J. The failure of the oesophageal groove reflex, when

376   fluids are given with an oesophageal feeder to newborn and young calves. Vet. Q.

377   1983,5:68-74.

378 [4] Swanson, E. W., Harris, J. D. Development of rumination in the young calf. J. Dairy Sci.

379   1958,41:1768-76.

380 [5] Borne, J. J. G. C. v. d., Alferink, S. J. J., Gerrits, W. J. J. Identifying ruminal drinking by

381   measurement of respiratory quotient and methane production in preruminant calves. J.

382   Anim. Sci. 2004,82 S1:365 (W209).

383 [6] Smith, B. P. Large animal internal medicine. 5th ed. St. Louis, Mo.: Mosby Elsevier;

384   2014.

385 [7] Akssenowa, M. J. Zur Physiologie des Magens der Wiederkäuer. Arch Tierernähr

386   Tierzucht. 1932,7:295-304.

387 [8] Flor, J., Linke, B., Sanftleben, P. Möglichkeiten der Computertomographie zur

388   Beurteilung der Mägenentwicklung beim Milchkalb. Züchtungskunde. 2012,84:213-

389   25.

390 [9] Jasper, J., Weary, D. M. Effects of ad libitum milk intake on dairy calves. J. Dairy Sci.

391   2002,85:3054-8.

392 [10] Appleby, M. C., Weary, D. M., Chua, B. Performance and feeding behaviour of calves

393   on ad libitum milk from artificial teats. Appl. Anim. Behav. Sci. 2001,74:191-201.

394 [11] Flower, F. C., Weary, D. M. Effects of early separation on the dairy cow and calf: 2.

395   Separation at 1 day and 2 weeks after birth. Appl. Anim. Behav. Sci. 2001,70:275-84.

396 [12] de Passillé, A. M., Marnet, P.-G., Lapierre, H., Rushen, J. Effects of twice-daily nursing

397   on milk ejection and milk yield during nursing and milking in dairy cows. J. Dairy Sci.

398   2008,91:1416–22.

399 [13] Khan, M. A., Weary, D. M., von Keyserlingk, M. A. G. Effects of milk ration on solid

400   feed intake, weaning, and performance in dairy heifers. J. Dairy Sci. 2011,94:1071-81.

401 [14] Overrein, H., Whist, A. C., Sølvberg, K. M., Nyhus, L. T. Godt kalveoppdrett – det er

402   bedre å bygge kalver enn å reparere kyr. Tine Rådgiving; 2015 [Norwegian].

403   [15] Nielsen, H. M., Olesen, I., Navrud, S., Kolstad, K., Amer, P. How to consider the value

404   of farm animals in breeding goals. A review of current status and future challenges. J.

405   Agric. Environ. Ethics. 2011,24:309-30.

406   [16] De Paula Vieira, A., Guesdon, V., de Passillé, A. M., von Keyserlingk, M. A. G., Weary,

407   D. M. Behavioural indicators of hunger in dairy calves. Appl. Anim. Behav. Sci.

408   2008,109:180-9.

409   [17] Phillipson, A. T. The movements of the puches of the stomach of sheep. Q. J. Exp.

410   Physiol. Cogn. Med. Sci. 1939,29:395-415.

411   [18] Constable, P. D., Wittek, T., Ahmed, A. F., Marshall, T. S., Sen, I, Nouri, M. Abomasal

412   pH and emptying rate in the calf and dairy cow and the effect of commonly

413   administered therapeutic agents. Proceedings of the 24th World Buiatrics Congress.

414   Nice 2006. p. 54-68.

415   [19] Jensen, M. B. The effects of feeding method, milk allowance and social factors on milk

416   feeding behaviour and cross-sucking in group housed dairy calves. Appl. Anim.

417   Behav. Sci. 2003,80:191-206.

418   [20] Lidfors, L., Isberg, L. Intersucking in dairy cattle - review and questionnaire. Appl.

419   Anim. Behav. Sci. 2003,80:207-31.

420   [21] de Passillé, A. M. Sucking motivation and related problems in calves. Appl. Anim.

421   Behav. Sci. 2001,72:175-87.

422   [22] Haley, D. B., Rushen, J., Duncan, I. J., Widowski, T. M., de Passillé, A. M. Effects of

423   resistance to milk flow and the provision of hay on nonnutritive sucking by dairy

424   calves. J. Dairy Sci. 1998,81:2165-72.

425   [23] Wise, G. H., Anderson, G. W., Linnerud, A. C. Relationship of milk intake by sucking

426   and by drinking to reticular-groove reactions and ingestion behavior in calves. J. Dairy

427   Sci. 1984,67:1983-92.

428   [24] Bourne, D. Assessment of pain in ruminants. In: Wildpro - the electronic encyclopaedia

429   and library for wildlife.

430   http://wildpro.twycrosszoo.org/S/00Man/PainRumOverviews/PainGeneral/02Assessm

431   ent_PainRum.htm. 2015.

432   [25] Dewi, S., Kristiansen, V., Lindkær-Jensen, S., Larsen, S. Between- and within-patient n-

433   level response surface pathway design in dose-finding studies. Open Access J. Clini.

434   Trials. 2014,6:63-74.

435   [26] Altman, D. G. Practical statistics for medical research. London: Chapman and Hall;

436   1990.

437   [27] Agresti, A., Franklin, C. A. Statistics: the art and science of learning from data. Upper

438   Saddle River, NJ: Pearson Prentice Hall; 2012.

439   [28] Dewi, S., Aune, T., Bunæs, J. A., Smith, A. J., Larsen, S. The development of response

440   surface pathway design to reduce animal numbers in toxicity studies. BMC

441   Pharmacol. Toxicol. 2014,15:18.

442   [29] Uys, J. L., Lourens, D. C., Thompson, P. N. The effect of unrestricted milk feeding on

443   the growth and health of Jersey calves. J. S. Afr. Vet. Assoc. 2011,82:47-52.

444   [30] Bach, A., Domingo, L., Montoro, C., Terré, M. Insulin responsiveness is affected by the

445   level of milk replacer offered to young calves. J. Dairy Sci. 2013,96:4634-7.

446   [31] Schingoethe, D. J., Casper, D. P., Drackley, J. K., Ludens, F. C. Increased solids intake

447   and feeding frequency for calves in hutches during cold weather. J. Dairy Sci.

448   1986,69:1063-9.

449   [32] Kmicikewycz, A. D., da Silva, D., Litherland, N. B. Effects of a modified intensive milk

450   replacer program fed two or four times daily on nutrient intake, calf growth and

451   health. Minnesota Dairy Health Conference. St. Paul 2010. p. 95-6.

452   [33] Thomas, T. J., Weary, D. M., Appleby, M. C. Newborn and 5-week-old calves vocalize

453   in response to milk deprivation. Appl. Anim. Behav. Sci. 2001,74:165-73.