

Development of a welfare assessment protocol for dairy calves from birth through to weaning

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Abstract

The aim of this study was to develop a welfare assessment protocol using different indicators, for pre-weaned dairy calves, that is feasible and time efficient. To this end, the protocol had to combine animal-based indicators (measurements on physiology, general appearance and behaviour) providing the basis for welfare assessment, with resource-based indicators (measurements on management and the environment) providing the basis for identifying risk factors. Indicators, both animal- and resource-based, were selected by a review of existing literature and a process of expert consultation. Following the formulation phase, the protocol was then applied on five Irish dairy farms to develop further for completeness and on-farm feasibility. After each on-farm application, the protocol was critically evaluated, and modifications were made accordingly. Upon completion of the on-farm application phase, a feasible, reliable and time-efficient protocol was produced.

Keywords: animal welfare, behaviour, dairy calves, health, housing, management

Introduction

Animal production systems are continually evolving in an effort to improve production efficiency and meet increasing demand for animal-derived protein (Thornton 2010; Boland *et al* 2013). Evolution of animal production, however, has not always been in sync with animal welfare. In the dairy industry, for example, intense selection for milk production resulted in declining fertility, and increasing rates of lameness and metabolic disorders (Webster 2005; Rauw 2009; Oltenacu & Broom 2010). Welfare assessments are required to identify potential risk factors of diminished animal welfare (Lundborg *et al* 2005; Brscic *et al* 2012; Leruste *et al* 2014) in evolving production systems. These can be carried out on-farm, through human application and technological applications using sensor data or vision technology. Potential technological applications include using 3D imagery to detect lameness, or thermal imagery to detect mastitis (Hovinen *et al* 2008; Song *et al* 2008; Rushen *et al* 2012; Viazzi *et al* 2014). Evaluation of routinely collected herd data, such as mortality, productivity, and fertility parameters, can also be used to assess welfare. Mortality rates provide a good starting point for identification of physiological health issues as these rates can be reflective of basic herd-level health and functioning (Ortiz-Pelaez *et al* 2008; De Vries *et al* 2011), however mortality has limited meaning with respect to animal welfare (Ortiz-Pelaez *et al* 2008). In North America and some European countries, calf

mortality rates of $\geq 10\%$ have been identified (Compton *et al* 2017). Differences have also been identified in mortality rates based on gender, with rates of mortality approximately 40% higher among male calves compared to females in commercial Irish dairy herds (Department of Agriculture, Food and the Marine [DAFM] 2013, 2014, 2015).

Although using routinely collected data to estimate welfare has some merit (Sandgren *et al* 2009; De Vries *et al* 2014; Parker Gaddis *et al* 2016), on-farm assessments remain necessary for confirmatory purposes, for conducting other welfare measurements that would not be routinely collected (eg behavioural observations), but also due to discrepancies in data recording. Such discrepancies could exist as a result of unintentional errors in the recorded data, or manipulation of data, in an attempt to conceal welfare issues, enhance breeding values, or to avoid inspections, or potential financial penalties to subsidies received. Such penalties may arise from having elevated values for parameters, such as calving difficulty or mortality rates. Routinely collected data often focus on performance traits, such as herd fertility or herd productivity, and as welfare is determined by factors beyond that of physical performance, this limits the capabilities of routine herd data to provide an indication of welfare. Traditionally, on-farm welfare assessments for dairy cattle, such as the 'Animal Needs Index', have used mainly resource-based (management and environment) indicators to assess welfare (Bartussek *et al* 2000). These assessments

cannot accurately differentiate welfare of animals exposed to similar management and environments and therefore do not provide an adequate evaluation of welfare status. To assess welfare more accurately, animal-based indicators, such as physiological health parameters, general appearance assessment, and behavioural measurements should represent the majority of the protocol (Webster *et al* 2004; Fraser 2008). The EU-funded Welfare Quality® project commenced in 2004 with the aim of developing standardised systems for assessing animal welfare using mainly animal-based indicators. In this project an assessment protocol for dairy cows was developed that requires a considerable amount of time to implement (6 h for one person to apply to a 60-cow herd) due to a large number of animal-based indicators, 43 in total, in the protocol (De Vries *et al* 2013). Hence, the Welfare Quality® protocol for dairy cows lacked feasibility and therefore has limited practical use (Knierim & Winckler 2009; Blokhuis *et al* 2010; De Vries *et al* 2014). It is therefore imperative that any protocols developed and implemented to assess animal welfare be balanced for application time, feasibility and assessment accuracy.

While assessment protocols for a wide range of animals were produced during the Welfare Quality® project, a protocol for dairy calves (male and female) was not developed. Existing protocols for dairy calves tend to focus on female calves, using indicators from a single-category (animal-, environment- or management-based indicators) (Whay *et al* 2003; Vasseur *et al* 2012). Single-category assessments can create analysis constraints; for example, assessment focusing solely on animal-based indicators do not allow for risk factor analysis. By combining indicators from all three categories valid assessments can be made which, when applied on a large number of farms, could also allow for identification of potential risk factors.

The objective of this study, therefore, was to develop a reliable, feasible and time-efficient welfare assessment protocol, using different indicators, for male and female dairy calves, during the pre-weaning and weaning phases (defined as beginning on day one of life to the point when milk feeding has ceased).

Development of the protocol consisted of two phases. Phase 1, the formulation phase, included the following steps: i) formulation and structuring of an interview for the calf manager, to assess management practices; ii) selection of environmental measurements and observations; and iii) selection of animal-based welfare indicators and appropriate scoring systems. Phase 2 included on-farm evaluation and further development of the protocol, conducted on commercial Irish dairy farms.

Formulation phase

Interview: self-reports of management practices

Management is a key determinant of welfare status (Fraser 2008; Cummins *et al* 2016). To identify management practices already applied within the herd, an interview was developed to be carried out face-to-face with the principal calf manager. Key management areas directly related to calf

welfare were selected based on a comprehensive review of literature in this field, and a process of consultation with experts in the fields of animal welfare and welfare assessment, as well as a number of farm managers. Areas addressed included colostrum management and post-colostrum feeding (Godden *et al* 2009), morbidity and mortality (Uetake 2013), health treatments administered, hygiene (Lehenbauer 2014), and weaning methods (Weary *et al* 2008). In addition, a postal survey by Cummins *et al* (2016) investigating management practices on spring calving dairy farms was reviewed and a number of questions, relevant to the interview, were selected and adapted to an interview style. The interview consisted of 60 questions, which followed the natural sequence of processes in a calf management system from birth through to weaning. Where relevant, questions differentiated between management of male and female calves, and between calves born within the hours of a working day (0600–2000h) and at night (2000–0600h). Subjects covered during the interview, and justification for their inclusion, are given in Table 1.

In addition to data collected by interviewing the calf manager, further information was available, with herd owners' consent, from the Irish Cattle Breeding Federation (ICBF) HerdPlus®; this provided information on calf mortality rates and stillbirths, calf date of birth, breed, sex, sire and economic breeding index value.

Assessment of the environment

Housing facilities and the environment calves experience can have a pronounced effect on welfare (Peña *et al* 2016). For this reason, assessment of environmental conditions was an essential part of this protocol and could be used to identify risk factors. Environmental measurements pertaining to space allowance (Jensen & Kyhn 2000; Tapki *et al* 2006), temperature and wind speeds within and outside the calf house (Bokkers & Koene 2001; Peña *et al* 2016), hygiene conditions (Ridge *et al* 2005; De Waele *et al* 2010) as well as feed and water supply (Coverdale *et al* 2004; Porter *et al* 2007), were included in the protocol. Selection of indicators was based upon: i) correlation with health and welfare outcomes in the literature; and ii) feasibility and reliability of recording.

To gain an insight into space allowance per calf, dimensions of all group and individual pens, and number of animals present were included in the protocol. Adequate ventilation and air capacity in a calf house is important to prevent respiratory issues (Bryson 1985; Sivula *et al* 1996). Measurements of length and breadth of the calf house and height at the roof eaves and ridge were included to allow cubic air capacity of the house to be calculated. Type of bedding material, and average depth of bedding in each pen were incorporated into the environmental assessment, as this can influence welfare, by affecting physiological health and animal comfort (Panivivat *et al* 2004). To assess cleanliness and dryness of bedding material, a scoring system adapted from Lundborg *et al* (2005) was used (Table 2).

Ammonia gas concentrations at elevated levels in animal housing facilities can cause ocular and respiratory tract irri-

Table 1 Subjects, and topics, addressed during the interview with the principal calf manager and justification for addressing these areas.

Subject	Topic	Justification
General information	Herd size	Gulliksen <i>et al</i> (2009)
	Herd health	Mallard <i>et al</i> (1998)
	Vaccinations	Meganck <i>et al</i> (2015)
Colostrum management	Time from parturition to first milking	Conneely <i>et al</i> (2013); Moore <i>et al</i> (2005)
	Timing of colostrum feeding	Jaster (2005)
	Volume of colostrum provided	Jaster (2005); Beam <i>et al</i> (2009); Godden <i>et al</i> (2009)
	Colostrum feeding method	Besser <i>et al</i> (1991); Godden <i>et al</i> (2009)
Post-colostrum feeding	Transition milk feeding	Godden <i>et al</i> (2009)
	Volume of milk provided	Jasper & Weary (2002); Quigley <i>et al</i> (2006); Vieira <i>et al</i> (2008); Soberon <i>et al</i> (2012)
	Grouping of calves	Vieira <i>et al</i> (2008)
	Feeding method	Hammell <i>et al</i> (1988); Jensen & Budde (2006)
Health and treatments	Morbidity	Johnson <i>et al</i> (2017); Jorgensen <i>et al</i> (2017)
	Mortality	Windeyer <i>et al</i> (2014)
	Treatments	Sayers <i>et al</i> (2016)
Hygiene	Cleaning procedures	Phipps <i>et al</i> (2016)
	Cleaning frequency	Klein-Jöbstl <i>et al</i> (2014)
Weaning	Weaning method	Weary <i>et al</i> (2008)
	Weaning age	Eckert <i>et al</i> (2015)

tation in animals (Urbain *et al* 1996; Lundborg *et al* 2005). Assessment of ammonia gas concentrations at bedding level were incorporated (Gastec ammonia tubes and the Gastec GV-100S pump, Envirosafe Ireland, Wicklow, Republic of Ireland). To maintain feasibility, measurements would be taken in at least two pens with representative bedding conditions observed on the farm and at least at one location per pen (eg centre of the pen). High ammonia concentrations occur as a result of the presence of faeces and urine and can therefore be used to assess bedding hygiene in combination with other measurements.

Climatic conditions, such as temperature and wind velocity, can affect thermoregulation of calves and therefore influence calf welfare (Lago *et al* 2006; Norton *et al* 2010). To assess the microclimate to which calves were exposed, ambient temperature and wind speed were included. Although ambient temperature and wind velocity might differ between pens, it would be measured at least once in a central location of the calf house which is deemed representative of that experienced in the house, and also outdoors in a clear, open area, as a reference. Temperature data loggers are ideal for this purpose. Wind speed would be measured at calf level (0.75 m above ground level) in at least one location within the housing facility using an anemometer

(Airflow TA-2 anemometer, Airflow Developments Ltd, High Wycombe, UK). A wind velocity measurement would also be recorded externally, as a reference.

In calf-rearing systems, hygiene can have an impact on physiological health and therefore on welfare (Dewes & Goodall 1995; Al Mawly *et al* 2015). To assess cleaning routines, and verify answers given during the interview, an assessment of hygiene of feeding implements, such as stomach tubes used for feeding newborn calves, and colostrum collection containers, was included. Hygiene would be assessed using commercial test kits, such as 3M™ Clean-Trace surface protein plus test kits (3M™, Minnesota, USA). These kits measure the presence of proteins, such as milk residues and biological contaminants (bacteria, fungi), to verify effectiveness of cleaning routines. This is a semi-quantitative assay where colour formation indicates surface hygiene on a four-point reference scale, yielding a result within 15 min of testing. This provides an assessment of cleaning routines, which is validated (Simpson *et al* 2006), quick, feasible and easy to use. This assessment will then allow for differentiation between farms, cleaning methods (eg use of cold versus hot water, as determined during the interview), and feeding implements.

Table 2 Assessment of bedding material on hygiene and moisture content, using a scoring system of zero to three, zero representing clean/dry and three representing soiled/wet.

Score	Hygiene description	Moisture description
0	Fresh/clean	Dry/fresh
0.5	75% clean	Wet at perimeter
1	Slightly dirty	Slightly damp
1.5	Moderately dirty	Wet in spots
2	Dirty	Wet
2.5	Very dirty	Very wet
3	Extremely dirty/wet	Extremely wet

Solid feed intake rates begin to increase from 14 days of age, when offered, and promote rumen development through anaerobic fermentation of feed particles, as well as development of absorption mechanisms (see Khan *et al* 2011). Availability of solid feed is particularly important at weaning to address the energy deficit arising from milk removal. Offering solid feed pre-weaning can therefore influence calf welfare by reducing weight loss and stress experienced in this dietary transition period (see Khan *et al* 2011). Availability of concentrates and roughage, such as hay or straw, in each pen was recorded. Provision of fresh drinking water and method by which it is provided in each pen was also recorded. Calving pen use, and design, can influence calf welfare (Vasseur *et al* 2010), therefore, observations on calving pens were included: floor type classification (solid concrete, concrete slats etc), number of calving pens, distance from calving pens to calf house, and if air space is shared between calving pens and calf houses. In the calf house, observations regarding how animals are penned (number of pens, number of calves per pen, mixed sex or single sex groups etc), and highest and lowest identification number of calves in each group should be recorded to determine maximum age differences within groups, once date of birth is accurately recorded, as is a mandatory legislative requirement in the Republic of Ireland.

Assessment of animals

Assessment of clinical health, physiology and behaviour, requires animal-based measurements, some of which are directly related to management and environmental conditions (Cummins *et al* 2016). Clinical health is one of the major aspects of welfare, and can be quickly assessed using visual indicators, such as demeanour, faecal consistency and respiration rate. Clinical health of all calves would be assessed using a visual health scoring system which was developed as part of this protocol by combining and modifying systems developed by the School of Veterinary Medicine, University of Wisconsin-Madison (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf) and Teagasc (Sayers *et al* 2016) (Table 3). Preferably all calves in the herd should be

assessed with this clinical health protocol, for a more definitive assessment of welfare.

Colostrum provides a source of passive immunity to the immunologically naïve calf, and the importance of colostrum quality and its association with calf health have been well documented (Robison *et al* 1988; Godden *et al* 2009; Furman-Fratczak *et al* 2011). Fresh colostrum samples, from ≥ 6 cows, should be collected where possible, to investigate the quality of colostrum produced by cows in the herd. In studies investigating colostrum quality on commercial farms, the number of samples collected per farm varied. A survey assessing colostrum quality on dairy farms in Pennsylvania collected a single colostrum sample (produced by a single cow) on 55 different farms (Kehoe *et al* 2007). A larger study investigating colostrum quality in the United States collected 827 colostrum samples from 67 dairy farms, equating to approximately 12 samples per farm (Morrill *et al* 2012). In this study, however, herd sizes ranged from 70 to 500 cows. Given the relatively small average herd size in Ireland, (70 cows; Irish Cattle Breeding Federation [ICBF], personal correspondence 2018) it was decided that a minimum of six samples from six individual cows should be collected. In situations where large herd sizes are being assessed, the minimum sample requirement should be increased, to approximately 10% of the herd. These can then be analysed in duplicate, to determine immunoglobulin G (IgG) concentration, using radial immunodiffusion kits (Triple J Farms, Bellingham, Washington, USA), which are considered the gold standard for quantifying IgG in bovine serum and colostrum (Weaver *et al* 2000; Biemann *et al* 2010).

Calf blood serum IgG concentrations are indicative of colostrum management, passive immunity, and potential risk of morbidity and mortality (Godden *et al* 2009). The collection of blood samples from calves ≤ 6 days old (Tyler *et al* 1996) will indicate if calves have achieved adequate transfer of immunity, but also if practices relating to colostrum management outlined in the interview are being implemented. The number of calves ≤ 6 days of age will vary depending on factors, such as herd size, timing of visit within the calving season, and compactness of calving, therefore a minimum sample number is not described. To collect blood samples, subject to legislative approval, the jugular vein can be used as other sampling sites, such as the coccygeal artery, can be small and difficult to isolate in calves (Adams *et al* 1991). The collected blood samples should be kept on ice until refrigerated at 4°C for 24 h prior to serum separation by centrifugation (3,000 g \times 30 mins) at 4°C. Following centrifugation, serum samples can be frozen at -20°C until IgG concentration determination.

Collection of faecal samples will allow for the presence of pathogens commonly associated with calf enteritis, such as *Escherichia coli*, *Cryptosporidium parvum*, rotavirus and coronavirus (Meganck *et al* 2015). Fresh faecal samples were collected using faecal containers (Sarstedt AG & Co, Germany). Faecal sample analysis can be conducted using vertical flow immunochromatography test kits (Bio-X Diagnostics SA, Belgium).

Table 3 Definition of and scoring levels used in the health scoring system, adapted from the School of Veterinary Medicine, University of Wisconsin-Madison and Teagasc (Sayers *et al* 2016) to assess dairy calf health in the welfare protocol.

Indicator	Definition	Scoring	Score levels
Demeanour	Combined evaluation of behaviour and responsiveness	3-point scale of 0 to 2	0 = Bright, alert, responsive 1 = Dull, possibly depressed, less responsive 2 = Dull, apathetic, unresponsive
Mobility	Ability to stand unassisted and move freely	3-point scale of 0 to 2	0 = Stands unassisted, actively mobile 1 = Slow to stand, limited mobility 2 = Assistance required to stand, no mobility
Cleanliness	Appearance and condition of each calf's coat of hair	3-point scale of 0 to 2	0 = Clean, dry, shiny coat 1 = Slightly dirty, dull coat 2 = Dirty, matted, dull coat
Lesions/wounds	Any damage or abrasions to skin surfaces	Yes/No. If yes, location of lesions/wounds recorded	Yes, visible lesions/wounds No, absence of lesions/wounds
Ears	Positioning and activity of ears	3-point scale of 0 to 2	0 = Upright, alert, mobile 1 = Slightly drooped, active 2 = Drooped and limp
Nasal	Presence of any mucous discharge from nasal passages	3-point scale of 0 to 2	0 = Clear, discharge free 1 = Small amount of cloudy mucous visible 2 = Excessive bilateral mucous discharge
Eye	Position, appearance and presence of ocular discharge	3-point scale of 0 to 2	0 = Bright, pronounced 1 = Slightly dull, presence of discharge 2 = Dull, sunken discharge visible
Cough	Presence of a cough, increased respiratory rate	3-point scale of 0 to 2	0 = Normal breathing 1 = Spontaneous cough 2 = Continuous cough, increased respiration
Navel	Evidence of navel infection	3-point scale of 0 to 2	0 = Normal, pain free to handle 1 = Slightly swollen, tender 2 = Swelling, inflammation of navel area
Faecal consistency	Evaluation of faecal density/viscosity	3-point scale of 0 to 2	0 = Normal, solid 1 = Semi-formed, paste-like 2 = Watery fluid
External parasites	Presence of external parasites, such as lice, mites	Yes/No	Yes, parasite visible on skin/in coat No, skin/hair free from external parasites
Ringworm	Evidence of fungal infection on skin	Yes/No	Yes, hair loss, skin lesions No, normal hair coat and skin

Table 4 Ethogram, adapted from de Wilt (1985), which categorises and defines various behaviours, used for behavioural observations in the welfare protocol.

Category of behaviour	Behaviour	Definition
Posture	Standing	Calf is standing
	Lying	Calf is lying/resting
General	Walking	Calf is walking
	Not visible	Behaviour of the calf is not visible
	Other	Events not reflective of welfare status (eg defaecates or urinates)
Feeding behaviour	Drinking milk	Calf is drinking from a bucket, trough, teat or automatic feeding station
	Drinking water	Calf is drinking water
	Eating	Calf eats concentrates or roughage, or other solid feed (proximity of head to feed)
	Ruminating/chewing	Calf is chewing
Comfort behaviour	Grooming	Calf licks itself, including snout/nose licking
	Scratching/rubbing/stretching	Calf scratches itself with one of the legs (generally hind legs) Calf rubs itself on pen structure Calf stretches itself
Abnormal behaviour	Tongue playing/rolling	Calf makes repeated movements with its tongue, inside or outside mouth
	Urine drinking/oral manipulate prepuce	Calf drinks the urine of another calf Calf attempts to suck the navel area of another calf
	Orally manipulating pen structure	Calf licks, nibbles, sucks or bites at the pen structure (barriers, walls, buckets, troughs, etc)
Play behaviour	Play behaviour/mounting/head-butting	Calf runs, jumps, changes direction suddenly, bucks, kicks hind legs, twists or rotates body Calf mounts or attempts to mount a pen-mate
		Calf is engaged in head-to-head pushing with another calf
Social behaviour	Social interaction	Calf licks another calf in the same area multiple times Calf nibbles, sucks or bites at another calf

Behaviour is an important indicator of welfare: for example, play behaviour is generally associated with good welfare, while high levels of oral manipulation of pen-mates, or the pen structure, can be indicative of a welfare issue (Margerison *et al* 2003; Krachun *et al* 2010). To minimise time required on-farm, and maintain assessment feasibility, indirect behavioural observations can be made from video recordings taken during the farm visit. Based on consultation with experts in the field of animal welfare, a recording period of 60 min was deemed sufficient to have an impression for normal and abnormal behavioural patterns and to be able to compare behaviour of calves between farms. Recordings of two group pens, of contrasting composition (ie male and female calves), or if male and female calves were grouped together, then a group of older calves (> 3 weeks old) and a younger group (< 3 weeks old) would be assessed, to provide

a reflection of the situation on that particular farm. To ensure consistency across behavioural observation conditions, recordings should be made during daylight hours, as was carried out during the on-farm development phase.

Once the groups were selected, video cameras (GoPro Inc, CA, USA) were set-up to capture a clear and unobstructed image of the entire pen area, within which the activities of calves could be identified. Prior to recording, any lighting available must be switched on to ensure maximum visibility in the recordings. Recordings can then be scored at a later date using the ethogram as outlined in Table 4, which is adapted from De Wilt (1985). Behaviours would be observed by scan sampling at 5-min intervals (± 30 s) for the duration of the 60-min recording. During the interview, feeding times would be determined in order to relate observed behaviour to proximity of feeding times. Observations would be limited to

two groups due to availability of recording equipment; however, observations on a greater number of groups should be conducted where possible. This should not influence assessment feasibility as setting up of recording equipment has a relatively small time requirement.

Observers

Application of the protocol requires two persons due to the inclusion of blood sampling and heart girth measurements, which require one person to restrain the animal while the second collects the sample/measurement. The remaining components of the protocol (interview, observations, etc) can be distributed between the two persons to reduce the time requirement of the protocol. A trained and experienced individual should conduct the interview, health scoring and environmental observations (eg bedding assessment) on each protocol application, while the second person, who has received basic training, organises the video-recording and carries out the environmental measurements (wind speed, ammonia measurements). This separation of tasks could serve to eliminate variation in measurements (eg bedding assessment, health scores, and interview application) due to differences between observers.

On-farm development phase

Ethical approval was received from the Teagasc Animal Ethics Committee (TAEC) (TAEC102/2015) and procedure authorisation (AE191132/P053) was granted by the Health Products Regulatory Authority (HPRA) of Ireland.

The on-farm development phase was carried out on five commercial Irish dairy farms between 1 and 16 December, 2016 to evaluate the processes involved and feasibility of the assessment. The number of farms in the on-farm development phase was based on 10% of a sample size required for a large scale study (Lackey & Wingate 1997), determined by a power calculation. Farms were selected on the criteria that they had a minimum of 50 autumn (September to December) calving cows, to ensure a minimum of 20 calves would be available when applying the protocol. Participation was on a voluntary basis and each farm was visited once. Herd owners were given three days' notice, in advance of the visit, informed of the tasks that would be carried out (interview, environment- and animal-based measurements) and the approximate duration of the visit (2.5 h).

Mean (\pm SD) herd size of the five farms was 302 (\pm 115.6) cows. One farm was 100% autumn calving, while the remaining four operated split-calving systems (a proportion of the herd calving in the spring 44.2 (\pm 25)% and the autumn 55.8 (\pm 25)%). The average autumn calving herd size was 137 cows, with an average calving season length of 12.8 (\pm 5.7) weeks.

The on-farm development phase allowed for identification of protocol strengths and weaknesses, which were then modified accordingly. These improved protocol clarity as well as allowing for a more accurate evaluation of calf welfare on commercial dairy farms.

Modifications to interview questions

In the interview process the following modifications were made during the course of the on-farm development phase to capture additional information and enhance the quality of information. In some instances, farmers provided valuable information which was outside the scope of the original question. Therefore, to ensure such information was collected each time the interview was conducted, additional relevant questions were included.

If disease testing of cows was conducted through bulk milk sample analysis, a follow-up question was included to determine which milk processor, or laboratory, was providing the service. Once known, the range of diseases screened for could be identified. Failure to conduct disease screening within a herd, and take appropriate action based on findings (eg culling infected cows etc), could result in an increased risk of calves being exposed to diseases early in life, but also *in utero* (Jawor *et al* 2007). Contract rearing involves the transfer of animals from the owner's farm to that of another party for rearing under contractual agreement. A question was also included in the general information section to investigate the use of contract rearing for heifer calves and the age at which calves move to contract rearing systems. This question was included to achieve a clear understanding of management systems on each farm and identify any movement of calves during the pre-weaning period.

On the topic of calf and colostrum management, further information was required on a number of specific areas. A question was included to determine the amount of time per day spent feeding and observing calves. This allowed for correlations between time dedicated to calves and calf welfare to be investigated, and also provided information on the labour requirements associated with calf rearing. Regular monitoring of cows approaching parturition is essential if difficult calvings are to be lessened and prompt provision of colostrum achieved. Use of calving cameras and frequency of physical observation of cows at night was therefore investigated to allow risk factors for poor calf welfare to be assessed. When revising the assessment protocol, one of the weaknesses identified was the lack of information on factors affecting adequate passive transfer (APT) of immunity in calves. The importance of good colostrum management in achieving APT is well-established, however evidence exists that in the Republic of Ireland for example, on-farm management is sub-optimum (All Island Disease Surveillance Report 2016; Cummins *et al* 2016). In light of this, a particular emphasis was placed on assessment of colostrum management practices. In the originally formulated interview, colostrum management areas addressed included timing from parturition to first milking of the cow, method and timing for feeding colostrum, and if and how colostrum was heated prior to feeding. During the pilot study, however, it was decided to include additional questions to determine if and how colostrum quality was assessed prior to feeding, if colostrum was pooled for feeding, if freshly calved cows were grouped

together and milking frequency applied. This information would allow for risk factors associated with achieving APT in calves to be identified, both within and between herds, in conjunction with collection and analysis of blood samples.

On occasions during the on-farm development phase, additional information of value was voluntarily provided by interviewees in the areas of post-colostrum feeding and management. To ensure this additional information was collected in future interviews, specific questions related to these areas were formulated and included in the interview. These questions addressed the source of transitional milk used (eg calves' own dam, or combined from a number of cows), use of individual calf pens, duration spent in these pens and method of feeding calves in these pens. If computerised feeding systems were used, follow-up questions were included to determine the age at which calves were introduced to the feeder, and details of the feeding programme applied. When investigating the frequency with which feeding equipment was cleaned, an additional question was included to determine how thoroughly the equipment was cleaned (eg using cold water only, or warm water and a detergent) on each occasion, as this could influence the spread of infectious disease.

When collecting information on health and treatments, a number of questions were re-designed to improve clarity and ensure there was no ambiguity surrounding any questions. Also, the prevalence, cause and method of diagnosis of calf pneumonia were investigated through the inclusion of additional questions. For sick calves placed in isolation, a follow-up question was included to determine where they were re-located to once recovered, as re-introduction to their original group could be a potential risk factor to calf health (Lundborg *et al* 2005), however such calves should enter a group to allow for social interaction.

Carry over of infectious agents from one year to the next can occur if facilities are not sufficiently cleaned in the intervening period. On the subject of hygiene practices, a question was therefore included to determine how (eg bedding removed, washed and disinfected, or bedding removed only) and when calving pens and calf pens were cleaned once the calving season was completed.

To gain a clear understanding of weaning practices, additional information was sought on weaning thresholds applied (eg age, bodyweight, concentrate intake etc). A question was also included to determine the age at which calves move outdoors to grass (eg prior to weaning, post-weaning, calendar date etc). When calves were being sold, a follow-up question was included to determine which specific types of calves are sold. This information allowed for differentiation of management, and health and welfare, of calves prior to sale to be compared to those remaining on the farm.

Following these modifications, the interview yielded clear and comprehensive information on how calves were managed, at all times differentiating between male and female calves and those born during the day and at night. Furthermore, verifying the accuracy of the information given during the interview was possible using results from environ-

ment- and animal-based measurements. Environmental measurements and observations proved successful, allowing within-farm evaluations to be made (eg bedding conditions of different pens) and also between farm comparisons.

Following application of the protocol and analysis of the data collected, herd owners should be furnished with a report in order to identify improvements which could be made on their farms. This should contain results on individual animal-measurements (eg colostrum quality for cows, serum IgG concentrations for calves, faecal sample results etc), together with the average figure within their herd, and recommended threshold values (eg colostrum IgG \geq 50 mg per ml). Information should also be provided on the environmental assessment (eg bedding score, ammonia concentration, space allowance etc) and recommended levels for each measurement. If the protocol is applied to a group of farms (eg in a large-scale study), group average figures could also be included in the report to allow farmers to compare the performance of their system to that of the group. Finally, the report should include expert-derived recommendations on improvement options. These farm-specific improvement options should be practical, feasible and must be communicated in a manner that is easy to follow, to allow for successful implementation.

During the on-farm development phase, the interview revealed contrasting management practices across the five farms indicating that the protocol is able to detect variation amongst farms. For example, on two of the five farms, colostrum was frozen for future use, while colostrum quality was only measured on one of the five farms. The mean mortality rate across the five farms (excluding stillbirths), identified through individual Herdplus® accounts, was 8.5%, which is similar to the national average of 6.1%: (DAFM 2015), and ranged from 3.4 to 11.9%. From calf manager statements, pneumonia and scours were identified as the main causes of calf illness, and four out of five farms reported experiencing issues with both on an annual basis. The specific cause of pneumonia was unknown in four out of five farms, while the cause of scours was known, from veterinary diagnosis, on three of the four farms that had experienced this health issue. Among the farms, feeding times, feeding method and volume of colostrum provided to bull and heifer calves were similar. For post-colostrum feeding, bull and heifer calves were subject to different management strategies, on four of the five farms. These included reduced feeding frequency and lower volumes provided to bull calves, and also differences in the type of milk provided. This demonstrates the importance and effectiveness of differentiating between sexes, and ages, as applied throughout the protocol. The results illustrate that within a small study population ($n = 5$), large variation existed in calf management practices applied.

Behavioural observations made through on-farm video recordings allowed for investigation of the presence of abnormal behaviours (eg tongue rolling, cross-suckling, etc), and comparisons of observed general behaviour patterns with those of expected behavioural patterns, based on existing literature. Using the ethogram, the types and frequencies of behaviours displayed by the observed groups

Table 5 Proportion of behaviours (average and range) observed at 5-min intervals (± 30 s) during a 60-min observation period of five pens of older, pre-weaned, calves (> 21 days; group 1) and five pens of younger calves (< 21 days; group 2).

Behaviour	Group 1		Group 2	
	Observation (%)	Range (%)	Observation (%)	Range (%)
Standing	35.50	21.70–58.40	34.21	33.30–48.30
Lying	52.72	33.40–77.98	59.25	50.00–82.10
Walking	0.83	*	0.26	
Eating	6.37	1.70–13.50	4.37	1.20–6.26
Drinking water	0.43		**	
Ruminating	1.08	1.10–3.20	0.71	1.04–1.80
Grooming	0.70	1.10–1.70		
Oral manipulating structure	0.42			
Scratching	1.25	1.70–2.70		
Chewing	0.15			
Social licking	0.30			
Tongue rolling			0.26	
Not visible	0.28		0.42	
Other			0.26	

* Behaviour observed in a single pen only, therefore, behaviour range is not available; ** Behaviour was not observed.

were identified by scan sampling (Table 5), allowing for behaviour at group level to be assessed. During the on-farm development phase the ethogram was altered slightly. The category 'urinating/defaecating' was removed, and replaced with 'other behaviour', to account for actions and bodily functions which are not related to welfare status. Lying was the most commonly observed behaviour and was witnessed more frequently among younger compared to older calf groups. This result is similar to the findings of Hänninen *et al* (2003) who noted it is the natural behaviour of neonatal calves to have a high resting frequency (20 h per day) which decreases with age. On two occasions humans were in the area where calves were contained for part of the recording period. For these groups, increased activity levels were witnessed in the behavioural observations, potentially due to human presence. Based on this, it was established that to prevent calf behaviour being influenced in any way, recordings must not commence until all individuals have vacated the area. This was successfully achieved by communicating clearly to all persons present on the farm to refrain from entering the area until recording had ceased.

Blood sampling proved an important physiological measurement, providing information on animal health and colostrum management, and with a relatively small time requirement. As venepuncture sampling is invasive, approval must be granted from relevant authorities, and also from farmers, prior to application of the protocol. Internationally, this may not always be possible due to legal restrictions and cultural attitudes.

Discussion

By combining management-, environmental- and animal-based indicators, a welfare assessment protocol for dairy calves was successfully developed. On one occasion, during the on-farm development phase, it became apparent that answers provided during the interview were reflective of best practice, and not applied practice. This was only identified when animal- and environment-based indicators were undertaken, which illustrates the importance of on-farm assessment, and the potential pitfalls associated with the exclusive use of self-reports on management practices. The combination of indicators used in the protocol, therefore, ensures that an assessment of calf welfare, based on existing literature and previously validated measurements, can be made in a feasible and time-efficient manner, with little variation in application time based on herd size. Further improvements could be made to this protocol by validating measurements that were relevant for calf welfare, but for which limited evidence was found in the literature, for example, ammonia concentrations, and bedding material assessment.

To achieve protocol feasibility and time efficiency, the duration of the observation period per pen and the number of pens per farm was chosen to be limited; therefore, conclusions which can be made from the displayed behaviour are also limited. Although a longer observation period, for example 8 or 24 h (Jensen *et al* 1998; Jensen & Kyhn 2000), would provide more conclusive information on behaviour, it

would be less feasible and time efficient. The observation period chosen for this protocol, however, does provide an impression of group behaviour which can be referenced to expected behaviour, relative to proximity of feeding times. It can also be used to benchmark a group against other groups (male versus female) when enough farms are included in a study: and one farm against other farms. Behaviour observations can also be compared to other information collected; for example, feeding levels can be compared to frequency with which oral behaviours are displayed (cross-suckling, manipulating pen structure). By including the 1-h observation period, an impression of behaviour is achieved, without any negative impact on protocol feasibility.

To produce reliable assessments, measurements must be precise and accurate. For human observations, variation can exist in measurements, both within (intra) and between (inter) observers. To quantify, and minimise measurement variation with training, intra- and inter-observer reliability must be tested. During the on-farm development stage, observations were made by a single observer. Prior to this, the observer carried out bedding quality assessments, and clinical health assessments in two calf groups, twice per day, on three separate occasions, at Teagasc Moorepark, research farm. From these measurements intra-observer reliability was high (> 0.90 ; Cronbach's alpha). In cases where the protocol will be applied on a large number of farms, and more observers are needed to collect on-farm data, training would be required for all observers, and intra- and inter-observer reliability assessments needed to ensure consistent scoring.

This protocol draws on certain aspects of the work of the Welfare Quality® project and could potentially contribute to the recommendation that development and refinement of welfare assessment systems continue to be undertaken in the wake of the Welfare Quality® project (Blokhus *et al* 2010). While this protocol was developed in the Republic of Ireland, where seasonal calving is in operation, the indicators used are generic to calf welfare. By applying alterations, this protocol could be used internationally; for example, a number of questions in the interview would have to be re-designed to account for year-round calving systems. In the European Union, of which the Republic of Ireland is a member state, directives exist on farm animal welfare. Each member state must incorporate these directives into national legislation and ensure they are enforced. Invasive procedures for calves, such as disbudding, castration etc, are controlled under such legislation, and therefore were not assessed as part of this protocol. For non-EU member states and other international regions where invasive procedures are not regulated, it would be imperative that an assessment of such procedures is included as part of the welfare assessment. Areas of international application could include: i) in research to assess and compare production systems; ii) to improve dairy welfare standards; iii) in quality assurance schemes; and iv) as a basis for determining legislative compliance.

Protocol application required approximately 2.5 h, and as two persons were required, this equates to approximately 5 h in total spent on-farm. Given that a large quantity of data were collected in this period of time, 5 h was not overly

excessive, and less than that generally required for on-farm welfare assessments which include animal-based measurements (Knierim & Winckler 2009).

Through the combined use of previously validated animal, management and environmental measurements, a welfare assessment protocol for dairy calves was successfully developed. Following an on-farm application phase, the protocol proved feasible, providing a time- and cost-effective method of assessing welfare on commercial farms, particularly during periods of high labour demand. To achieve assessment feasibility, certain constraints were required which created assessment limitations. A 60-min observation period provided an indication of behaviour; however, a longer period of repeated observations would provide more conclusive information. The protocol is applied in a single visit, and while heart girths are used to estimate bodyweight, average daily gain (ADG) cannot be determined. Average daily gain would provide valuable information on animal performance and welfare; however, it would necessitate a minimum of two measurements at different time-points. To evaluate the success with which risk factors can be identified having applied the protocol, a large scale study would be required along with the associated statistical analysis of the data. This study was conducted as part of a larger project which aims to apply the protocol on a large number of farms at a later date.

In welfare assessments, classification systems are strongly desired by stakeholders, such as consumers, processors and government agencies, as it would allow them to rate and compare animal welfare standards among producers. This would indeed be invaluable, however, it is imperative that such systems are reliable and precise, to safeguard animal welfare and ensure all producers receive a result which is representative of welfare standards achieved. Attempts to reduce the associated subjectivity of overall welfare assessments have included development of ranking and weighting systems. Such systems would be transparent and standardised, and while the development of these systems proved successful, limitations to these systems have been identified.

Ranking systems involve positioning herds based on individual measurements, followed by producing an overall ranking (eg from best to worst) based on the sum of the individual measurements. This system has been considered by Whay *et al* (2003) and Webster (2005), and while it is clear and easy to understand, particularly for farmers, it is limited to standards within the observed population. For example, in a population where overall welfare standards are poor, some will inevitably rank highly and could be perceived as having good welfare standards.

A weighting system, which assigns values to measurements based on the associated impact on welfare, calculates an overall score as the sum of the weighted values. Such systems have been developed for animal welfare assessment (Bartussek 1999; Bracke *et al* 2002). A considerable limitation to this system is that compensation can occur as high scores in certain areas can offset low scores in others, giving an overall value which can be indicative of good to moderate welfare, which may not be the case. This has been

identified in a number of studies evaluating welfare classification (Botreau *et al* 2009; De Vries *et al* 2013; Sandøe *et al* 2017). Where a welfare classification system was developed for pregnant sows (Bracke *et al* 2002), the authors stated that it could not be determined at which point a change in 'welfare score' would equate to a definite change in welfare. This, along with other studies, highlights the level of variation which exists between outcomes of welfare classification systems and actual welfare status. The consensus among welfare classification studies is that compensation is extremely difficult to overcome in such systems, which not only results in inaccurate classifications but can also mask existing issues.

We believe that these limitations are due to the complex nature, and level of subjectivity associated with welfare appraisal, and not to errors or shortcomings on the part of those who have developed existing ranking/weighting systems. Therefore, we decided not to proceed with the development of a classification system, and instead focused on the individual measurements. This manuscript therefore describes how a feasible and reliable protocol was developed to assess different indicators of calf welfare; however, the scope of this study does not extend to that of classification of welfare based on protocol application. Any parties who wish to apply this protocol with a view to classifying welfare, should familiarise themselves with the advantages and limitations of different overall assessment methods, and then select, and develop, the most suitable system to use in conjunction with this protocol, based on their overall goal (ie ranking or comparing herds).

Conclusion

Through the combined use of management, environment and animal indicators, a reliable, feasible, and time-efficient dairy calf welfare assessment protocol was developed. By completing validation on a number of measurements, this protocol could be used for large scale welfare assessments and for identifying risk factors associated with dairy calf welfare.

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