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Research Reflection

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Methodology for experimental and observational animal studies in cow-calf contact systems

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Abstract

In this Research Reflection we describe a common standpoint on suitable methodology for controlled and observational studies in cow-calf contact systems in dairy production. Different methods to assess behaviour, health and production in cow-calf contact systems are outlined. Knowledge and experience from researchers working in this field supplement scientific literature whenever relevant. Specific methods including study design, early behaviour of cow and calf, social behaviour relevant to cow-calf contact systems, human-animal relationships and aspects related to management (milking, weaning and separation, health) are reviewed, and recommendations formed. We expect that this paper can contribute to a better understanding of the complexity of cow-calf contact systems and help to advance research in this area of dairy production.

Societal concerns about the traditional practice to separate the newborn dairy calf from the cow have stimulated various research questions on how to manage systems allowing cow-calf contact (CCC) and the animal welfare impact of these (for terminology see companion paper Sirovnik *et al*., 2020). CCC systems provide increased opportunities for the expression of natural behaviours, such as nursing and bonding, which is appealing from the animal welfare perspective (Gygax and Hillmann, 2018), and for the sustainability of the future dairy sector in terms of increased consumer trust (von Keyserlingk *et al*., 2013). However, keeping dairy cows and calves together has several challenges, and there is a need for research to address these.

To acknowledge these questions, formulate new ones and amplify collaboration, a European consortium of researchers with interest and experience in the field of cow-calf contact systems was formed. Discussions within the consortium made clear that standardization of experimental methods is needed in order to be able to make comparisons between studies and farm practices and draw clear conclusions on future research questions. Because there is little research in this area, knowledge and experience found in the consortium regarding management of CCC systems is summarized here. Thus, this position paper is based on previous published research as well as a compilation of knowledge and experience, and provides a starting point for new and further research in CCC. In a further companion paper in this issue, methods for studies of human attitudes in CCC systems is covered (Ferneborg *et al*., 2020). Here, we aim to describe and recommend current experimental and observational methodology to be applied in future CCC studies.

Getting started: study design, experimental planning and set up of CCC studies

The study design and methods used should facilitate future meta-analysis. Therefore, we encourage that the type of study and design should be specified.

Explanatory studies can be subdivided into experimental and observational studies (Dohoo *et al*., 2014). Experimental and observational studies are equally important when investigating CCC systems. On the one hand, experimental studies may be used to investigate the causality of specific factors while on the other, observational studies are important to find associations and generate risk and success factors that can better reflect the complex on-farm situation where multiple factors interact. Independent of type, research on CCC should describe the milk-feeding, weaning and separation phases (Sirovnik *et al.*, 2020). We suggest definitions of measures of production, health and behaviour used in experimental and observation CCC studies in Table 1.

Experimental studies

In experimental studies, the addition of control groups without cow-calf contact is strongly encouraged. Although not essential, it will aid comparison to studies on artificial rearing systems. We recommend that the CCC pairs are allocated within the first hours post-partum which will allow control animals to be separated directly after birth, if relevant for the research question. A parallel-group design, studying different treatments simultaneously, avoids confounding effects such as season, load of infectious disease or staff changes.

Conducting CCC over a period of at least two years would allow for testing individual cows as mothers as well as control cows (artificial rearing) applying a cross-over design, to control for the quality of individual cow's maternal care. However, such a cross-over design would require some cows to start as mothers and others as controls, and vice versa, and one needs to consider possible carry-over effects.

We suggest considering whether cow-calf pairs belonging to different CCC treatments should be kept together or in separate pens. Housing pairs from all treatments in the same pen(s) will improve the statistical power, useful when studying the effect of suckling on affiliative interactions, for instance. However, if different treatment groups are housed together in the same pen, effects may be diluted due to mutual influences such as disease transmission or social learning (Johnsen *et al.*, 2015a, 2015b, 2015c).

Observational studies

Observational studies are well suited to document and compare production parameters or other aspects of health and welfare in different CCC systems or with control systems (Hillmann *et al.*, 2019). Being based on commercial farms, external validity of results is high, however, research is limited to conditions and practices existing in the studied farms. Prospective cohort studies allow researchers to follow CCC and control animals over time may be valuable to assess long-term effects.

Practical and experimental design aspects to consider

Currently, only a few experimental stations or dairy farms are designed to conduct studies with CCC within the dairy barn. Here, we sum up some practical aspects to consider for new studies.

Barn design

Free-stall barns often have slatted floors designed for cows' claws, but unsuitable for calves. To avoid injuries, we recommend that calves meet cows only on slatted floors designed for calves, solid floors or on pasture. Younger calves prefer to rest close to their dam (Johnsen *et al.*, 2015a, 2015b, 2015c). To avoid competition for cubicles and consequential agonistic interactions caused by it with potential further negative consequences, cubicle-cow-ratio should be increased further when calves have access to the cows' lying area. A bedded head zone with ample space may serve as a resting area for calves. We encourage more studies on this topic.

The fixtures in a typical barn are constructed with respect to the size and strength of cows. Therefore, risks for the calves' health and safety should be identified and corrected. Calves should have access to a separate calf creep area inaccessible to cows for resting and provision of water, high quality roughage, concentrate and possibly additional milk or milk replacer. This separate area should align with the natural behaviour of group formation that happens after a calf is introduced to the herd and joins other calves in a kindergarten, known as a crèche. Once accustomed to this area, calves may be easily separated from the cows for example for examination or sampling, or for confinement while cows are being milked.

In barns with cow-driven automatic milking systems, different solutions for cow traffic might be tested, as well as the importance of resource distribution. Using selection gates for cows to access the calf will enable flexibility to test free, pre-milking and post-milking traffic in the same barn (Sirovnik *et al.*, 2020). Altering which resources that are shared between cow and calf may affect activity and movement within the system, as well as feed intake, milking frequency and resting patterns, making it crucial to clearly describe the traffic system.

Handling of animals

During the first days after calving, dams may be protective of their calf and may show aggressive behaviour towards people (Bouissou *et al.*, 2001). Special attention should be paid to safety precautions: calving pens can be equipped with 'squeeze-gates' for fast exit and self-locking racks, so that cows can be restrained when necessary. We recommend that cows that are aggressive towards staff for longer than 2 d post-partum, or ignore or are aggressive towards their calves, should be excluded from experiments for animal welfare and human safety reasons. However, these should be reported in publications to get information about the incidence of this behaviour in breeds and age cohorts.

If human contact does not confound the experimental design, it is recommended that CCC calves should receive gentle human contact (Lürzel *et al.*, 2015). Since CCC calves are not necessarily fed by human caretakers and may stay predominantly in the cow barn, there is a risk of CCC calves receiving less positive human contact and relatively more negative handling, for instance ear tagging and disbudding. The quality and quantity of human contact and the method of ensuring colostrum intake should be described and standardized across treatments, if relevant, since human contact in general and colostrum bottle feeding may affect the human directed behaviour later in life. Besides, the failure of passive transfer of IgG may affect health (Johnsen *et al.*, 2019). Should the cow contract a clinical disease necessitating medical treatment that might affect the calf, suckling should be paused or terminated. In every case, such events should be reported.

Cow behaviour before and during calving

To record behaviour before and during calving, early signs of upcoming calving need to be detected. During the last 12–24 h before parturition, the cow reduces social behaviour and may keep a distance to herd mates if the environment is spacious (reviewed by Rørvang et al. 2018). Under production conditions, dairy cows may also seek visual isolation from herd mates if given the opportunity (Proudfoot *et al.*, 2014).

Table 1. Definitions of measures used in experimental and observational animal studies in cow-calf contact systems

Production measures		Definition	References[a]
Milking	Machine milk yield	Milk yield obtained with milking machine	Bramley *et al.* (1992)
	Suckled milk yield	Measured or estimated milk yield that is being suckled by the calf	Beal *et al.* (1990), Rutledge *et al.* (1971)
	Total milk yield	The sum of machine milk yield and suckled milk yield	Bar-Peled *et al.* (1995)
	Strip milk	Milk obtained by hand milking or machine stripping after machine milking or suckling	Bramley *et al.* (1992)
	Residual milk	The milk remaining in the udder after milking/suckling and stripping. Requires exogenous oxytocin for removal	Bramley *et al.* (1992)
	Milking machine settings	Quarter or udder level milking, automatic stimulation, pulsation ratio and rate, vacuum level, automatic stripping and detachment level	Bramley *et al.* (1992)
	Milking routine	Duration and type of routines during milking, such as cleaning, pre-milking, pre-stimulation, strip milking	Bramley *et al.* (1992)
Calf total dry matter intake (DMI)	Calf solid feed intake	Total amount of consumption of concentrate intake and roughage intake	Roth *et al.* (2009)
	Calf milk intake	Total amount of milk intake; suckled milk intake or artificial milk intake, or both	
Body condition score (BCS)	Cows and calves	A numerical score describing energy reserves in the form of visible/palpable body fat and muscle tissues.	Edmondson *et al.* (1989)
Growth rate	Calves	The individual calfs' weight gain per time unit, or its growth in length and height per time unit. An indicator of body development and nutritional status	Roth *et al.* (2009)
Health status measures			
Calf diarrhoea (scours)	Calves	Enteric disease recognized by loose or watery stool and frequent defecation. Can have infectious or non-infectious cause. The general condition of the animal is more or less influenced depending on cause, severity and duration.	Roth *et al.* (2009)
Mortality rate	Cows and calves	Number of animals that die in a designated period divided by the number of animal-time units at risk during that period	
Respiratory disorders	Cows and calves	Pathological changes in the respiratory tract or symptoms thereof (fever, coughing, mucopurulent discharge) in nasal cavity, trachea, bronchiae or lungs	Roth *et al.* (2009)
Mastitis	Cows	Inflammation of the mammary gland	Andersen *et al.* (2010)
Somatic cell count in milk (SCC)	Cows	Mastitis indicator	IDF (2013)
Behavioural measures			
Cross-sucking (sucking)	Calves	Sucking of any part of another calf´s body, caused by the strong motivation to suck in relation to milk ingestion, and possibly reflecting frustration	Jensen (2003), Roth *et al.* (2009)
Intersuckling (intersucking)	Cows and calves	Sucking of udder area by other heifers or cows	Lidfors and Isberg (2003), Keil *et al.* (2000)
Cows and calves	Cows and calves	Suckling of a cow by a non-filial calf	Johnsen et al. (2015a, 2015b, 2015c)
Affiliative social interactions	Cows and calves	Social interactions related to cohesion of the group or individual; including allogrooming[a] (OR social licking)	Bouissou *et al.* (2001)
Allogrooming	Cows and calves	Social licking of mainly the head, neck and shoulder regions between cow-calf, calf-calf or cow-cow (excluding licking between adult animals in a sexual context)	Bouissou *et al.* (2001)
Agonistic social interactions	Cows and calves	Social interactions related to conflict and competition, including aggression (such as threats pushing, butting, fighting, chasing), avoidance and submission	Bouissou *et al.* (2001), Mills *et al.* (2010)

[a]This table is repeated in the online Supplementary File together with the cited references.

We recommend that CCC facilities should promote natural behaviours associated with calving both to detect upcoming parturition and to facilitate the formation of the cow-calf bond. We use the term bond to refer to the mutual, affiliative relationship between two individuals (i.e. between mother and infant) that lasts for a long time and survives temporary separation

(Newberry and Swanson, 2008). We propose a functional definition of bond (Sirovnik *et al.*, 2020) taking in account behavioural indicators to decide when a dam-calf pair is ready to be introduced into the herd.

Access to individual maternity pens that provide a visual barrier can help cows to find seclusion from other cows (Proudfoot *et al.*, 2014) and facilitate the intensive interaction during the first hours after calving, which is crucial in this early stage of bond development (von Keyserlingk and Weary, 2007). Besides, calving in a secluded and undisturbed area decreases occurrence of calves suckling alien cows (Jensen *et al.*, 2019).

Early behaviour of the calf

For research purposes, it is useful to characterize the different stages of the process from birth to first suckling to determine the vigour of the calf. We recommend focusing on landmark behaviours describing the 'vigour' of the calf: attempts to stand, body supported by two hooves, standing, walk, reach the udder, and suckle, with calves having shorter latencies described as having higher vigour (Barrier *et al.*, 2012).

The post-partum behaviour of the dam

During the first hour after calving, the cow licks her calf intensively, a behaviour that gradually declines during the subsequent hours (Jensen, 2012). This intense maternal care comes with a cost of reduced resting and feeding time. It is important to consider ways to facilitate an environment in which the cow can feel safe, rest, and eat well, as well as take care of her newborn. For instance, staying longer in the individual calving pen, or housing in small groups of cows for the first 2–3 weeks after calving, may facilitate resting and feeding in these animals. It was found that smaller groups (around 6 cows) resulted in lower competition levels compared to groups of 24 cows (Jensen and Proudfoot, 2017). In studies addressing maternal care and bonding, one should focus on pre-partum behaviour (seeking seclusion) and affiliative behaviours, including allogrooming, proximity and latency to reunite after a period (brief or long) of separation (von Keyserlingk and Weary, 2007; Rørvang *et al.*, 2018).

Studying social behaviour

The early social environment can affect later personality traits in different species, including cattle. First results suggest that CCC as compared to group housing of calves improves social skills and affects sociality and other personality traits in the short (Wagner *et al.*, 2013) and long term (Wagner *et al.*, 2012, 2015). Social behaviour and related personality traits can be studied in the home pen by observing social interactions in an undisturbed situation or responses to social challenge, such as regrouping or enhanced competition (Duve *et al.*, 2012). The whole range of agonistic and affiliative interactions should be included, but also spatial behaviour, which allows conclusions on sociality and social skills (Gibbons *et al.*, 2010). Individual testing outside the home pen can be standardized, and although methodology varies between studies, this type of social test falls into three main categories as listed below. All test situations outside the home pen have the disadvantage that other personality traits, especially fearfulness and coping style, interfere with social motivations and influence behavioural reactions.

A *social approach test/social reinstatement test* assesses social motivation and sociability by assessing the animal's latency to overcome a distance and rejoin one or more peers (Gibbons *et al.*, 2010). A *social skills test* assesses the appropriateness of an animal's responses during social encounters; the animal is individually confronted with one or more 'standard' animals and the social responses are observed. Appropriate responses are, for example, submissive behaviour as a response to a threat from a superior animal (Buchli *et al.*, 2017). An *isolation test* assesses the animal's fear and social reinstatement responses to social isolation, reflecting sociality, by measuring vocalization, exploration and locomotion (Wagner *et al.*, 2015). This is the least specific of the three types of tests, even when performed in familiar environments. It may be relevant to combine more tests or observations to get a broader picture of the effect of CCC on social behaviour and personality traits.

Studying human–animal relationship

The human-animal relationship (HAR) is the mutual perception of the animal and the human, based on previous interactions and reflected in their mutual behaviour (Waiblinger *et al.*, 2006). As described above, CCC systems may limit human contact during the rearing period to mainly negative interactions, which may negatively affect the animals' relationship to humans, both in the short and long-term (Lürzel *et al.*, 2015, Waiblinger *et al.*, 2020). To enhance our knowledge on these issues we need both experimental and observational studies on farm addressing HAR in CCC, since human-animal interactions differ considerably between farms. To get a complete picture, the human side should be investigated as well (see Ferneborg *et al.*, 2020). In any case handling procedures and amount and quality of human-animal contact should be described in detail. If the human-animal relationship is in focus, handling for other measures should be avoided, thus combinations of research questions need to be considered carefully.

Avoidance distance test

The avoidance distance test (ADT) in the barn has been validated at herd and individual levels for cattle of all ages (Lürzel *et al.*, 2016) and has shown good reliability (Windschnurer *et al.*, 2008). ADT at the feeding place showed somewhat lower validity (Waiblinger *et al.*, 2003). A precondition for a reliable and valid test is appropriate training of the experimenter with respect to distance assessment, standardized human behaviour and correct interpretation/estimation of withdrawal reactions of the animal. ADT can be performed in tie stalls and all loose housing systems, but it is important to opt for one location, be it barn (being most preferable), feeding place or pasture, as results are not directly comparable between different locations (Waiblinger *et al.*, 2003). The ADT can also be performed outside the home environment, mostly following a stationary person test. However, novelty and social separation trigger confounding factors; further pre-test handling and an eventual preceding test can affect the test results (Waiblinger *et al.*, 2006).

Stationary person test

Within the home environment, the stationary person test (SPT) has only been used at the herd level so far (Waiblinger *et al.*, 2003). Outside the home environment, the SPT is performed at the individual animal level, assessing the approach behaviour to a sitting or standing person. Confounding factors, as described for ADT outside the home pen, are even more relevant for SPT (Waiblinger *et al*., 2020). SPT seems worth doing in addition to the ADT (in the home pen) only if it is combined with other questions and respective tests, for instance regarding effects of CCC on personality traits, or if a deeper understanding of the AHR is searched for.

Studying production aspects

Milking

Calculation of milk yield for breeding indices is based on standardized milk recordings (ICAR, 2014). Nursing affects the records in various degrees, such as estimates of future milk yield. Since the 'real' total yield encompasses both the suckled milk and the saleable milk yield, some dairy farmers add an estimated amount of suckled milk when reporting to the recording association to avoid an artificial deflation of the herd milk yield. Full contact with calves causes unpredictable intervals of udder emptying. Thus, partial cow-calf contact systems with standardized suckling times might give more reliable milk production data and be preferred in the design of some studies. To document the natural variation in milk yields in a CCC system, both total and (in automatic milking systems) quarter level yields should be assessed if possible. Milk yield and composition (fat and protein content) pre and post separation as well as for the 305 d lactation should be registered.

CCC may negatively affect milk ejection during machine milking (Zipp *et al*., 2018). Alveolar milk ejection and milking performance are subject to many influences, e.g. milking routines (Weiss and Bruckmaier, 2005), milking equipment (Besier and Bruckmaier, 2016), HAR (Munksgaard *et al*., 2001) as well as the health status of the cow (Sandrucci *et al*., 2007). Studies addressing milk production in CCC systems should describe when and how cows are introduced to machine milking after calving, describing the milking process in detail (including if the calf was present or not). Besides milking and suckling times, we recommend a description of the type of milking (parlour or robotic system), milking frequency, the technical features of the milking machine, (such as liner design, automatic stimulation or stripping devices), the settings of the milking machine, (pulsation ratio, vacuum level, detachment level), and a description of the milking routine applied by the staff.

In case of investigating impaired milk let-down, any exogenous administration of oxytocin must be reported. The application of oxytocin delivers not only the part of milk that remained in the udder due to an impaired alveolar milk ejection but also the part of residual milk that is never accessible by common milking methods even if cows are properly stimulated before and during the milking process (Bruckmaier and Blum, 1998). This has to be taken into account when the amount of milk collected after exogenous oxytocin application is used to assess the difference in milk production of cows that nurse calves or are only machine milked.

Weaning

Weaning is the process with the end-result that the calf no longer drinks milk (Sirovnik *et al*., 2020). Early weaning implies that the calf is forced to transition from easily digestible milk to solid feeds, and to develop from a functionally monogastric animal into a ruminant. A sudden change in availability of milk may cause reduction in daily body weight gain and even weight loss (Veissier *et al*., 2013). Strong behavioural reactions due to hunger, frustration (and the loss of maternal care) are common, although the intensity may vary depending of age, body weight and the applied method (von Keyserlingk and Weary, 2007).

The age of the calf at which the weaning process starts and ends, whether weaning is abrupt or gradual, how a step-down of milk is managed, how the calf is kept, availability of water and solid feed such as concentrate, hay, silage and pasture, consumption of solid feed (alternatively time spent eating), and weight gain should be recorded. Behavioural reactions indicative of hunger should be recorded, including frequency and type of vocalizations, unrewarded visits at the milk feeder (Jensen and Holm, 2003) and sucking on interior or rubber teat, or other calves (Roth *et al*., 2009). The intake of solid feed and the daily gains should be documented, if possible beyond the day where milk is no longer available.

Separation

After a period of CCC, separation typically cause stress responses in both cow and calf. The type of separation should be described in detail (Sirovnik *et al*., 2020) as well as how and where the animals are kept after separation. Useful behavioural indicators in both cow and calves are frequency and type of vocalizations; high pitched (open mouth) is an especially valuable indicator of separation stress (Johnsen *et al*., 2015c) or low pitched (closed mouth); pacing along barrier, time spent standing and gazing in the direction of the calf (cow) (Johnsen *et al*., 2015a), with the head out of pen (Jensen *et al*., 2019), resting in recumbent position or calf restlessness. Other measures, such as faecal cortisol metabolites or salivary cortisol may be relevant. Calf weight gain and the intake of solid feed should be documented.

Separation stress can be alleviated if the calf's dependency on the dam for nutrition is reduced before separation (Johnsen *et al*., 2015a). For instance, teaching the calf to drink from a supplemental source of milk (an automatic milk feeder or nipple bottle) during the milk feeding period is a way to disentangle the effect of weaning off milk and the separation *per se*. Using the alternative source of milk, the calf can cover its nutritional needs at the time the dam is taken away. Research is needed to document the work-load associated with feeding supplemental milk. The intake of supplemental milk and solid feed reflects the degree of nutritional dependency of the dams' milk at the time of separation and should be documented, although this can be challenging to achieve.

Studies where suckling is prevented through an udder net or a nose flap should document occurrence of suckling attempts as a way to measure both the efficiency of the weaning methods and the behavioural response. Further, any side effects of nose flaps on solid food and water intake, as well as any injuries of the muzzle and nose should be reported.

Health status indicators

CCC systems may have an effect on health and production both in the shorter and/or the longer term. It is important to control for and document general health of both the herd and individual cows and calves. Results from regular health checks are recommended to be assessed in CCC experiments. The use of drugs is a parameter that should be included, however, it is important to note that the threshold for medication may vary between countries. Whenever possible, this threshold should be defined prior to the study (Roth *et al.*, 2009).

Cow health

There is a lack of information on the normal variation of somatic cell count (SCC) in CCC systems. It is known that SCC increases during the course of milking/suckling (Sarikaya and Bruckmaier, 2006). Therefore, suckling before sampling may interfere with the recorded result, and this artificial inflation may be misinterpreted as a symptom of subclinical mastitis. Due to the fact that the content of most of the milk components change during milking, the time of sampling and the sampled fraction should be reported in CCC studies focusing on udder health. Concerning subclinical mastitis incidences in CCC systems, outcomes of bacteriological examinations of milk samples might be more reliable than SCC measurements and thus should be reported whenever possible.

The fat to protein ratio in milk may be influenced by CCC to an unknown extent. For instance, a lower fat to protein ratio which indicates a higher risk of acidosis can be just the result of the disturbed milk let down during machine milking (Tancin *et al.*, 2007).

Future studies should address the influence of CCC on fertility measures and calving-related diseases, such as retained placenta.

Calf health

A dystocial birth may affect the ingestion and absorption of colostrum (Barrier *et al.*, 2013). The early ingestion of a sufficient quantity of high quality colostrum is a prerequisite for the calf to achieve passive immunity, which protects against infections. Failure of passive transfer is defined by calf serum IgG < 10 g/L at 24–48 h age, which may cause poor survival rates. Therefore, it is advisable to exclude cows and calves that have had a difficult delivery from CCC studies and ensure that pre-calving conditions are the same.

For diagnoses such as diarrhoea, information should be given on whether the general condition of the animal is also affected. For respiratory disorders, also, the general condition of the animals should be noted, and whenever the case is treated. Joint swellings/infections and umbilical infections should be noted (e.g. McGuirk and Peek, 2014). When evaluating calf health, it might be useful to record also some production parameters as an indirect effect of the health condition, such as daily weight gain (pre and post weaning), weight and body condition score at certain age (180 d is suggested), and intake of concentrates and roughage.

Using register data to measure long-term effects

To study long-term effects on health and production it is very useful to use centrally recorded health data where it exists. Small but important effects, such as the protein content of saleable milk, will be difficult to distinguish in short-term trials but can benefit from the large data sets. This is also the case for multifactorial conditions, such as impaired fertility or mastitis. A causal diagram should be constructed prior to the start of the study, in which suggested causal relations are established (Dohoo *et al.*, 2014).

Calf register data are generally less well recorded and are thus often less reliable than those for production cows, with the exception of mortality data. Unfortunately, mortality is recorded in many different ways and is not readily comparable across countries. Therefore, it is necessary to give information on how the mortality rate is calculated. We recommend that only live born calves are included, and that data on mortality age is given (Santman-Berends *et al.*, 2019).

In order to study the long-term effects on cows, it is crucial to note whether cows grew up in a CCC system, or if they were reared artificially. Regarding culling, both the age and causes for culling should be noted. For slaughter calves and bulls/bullocks, age and weight at slaughter should be registered, as well as carcass class/quality.

Conclusions

We have compiled research and practical experience on methodologies to make CCC studies more comparable and thus enable clearer interpretations of future CCC research. This includes factors to consider and report in order to facilitate future meta-analyses. More precise and detailed information on these aspects in future CCC studies will contribute to a better understanding of the complexity of CCC systems and help to advance this area in dairy production.

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