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**Assessment of individual- and group-level behavioral variation in dairy  
cattle – from personality to social networks**

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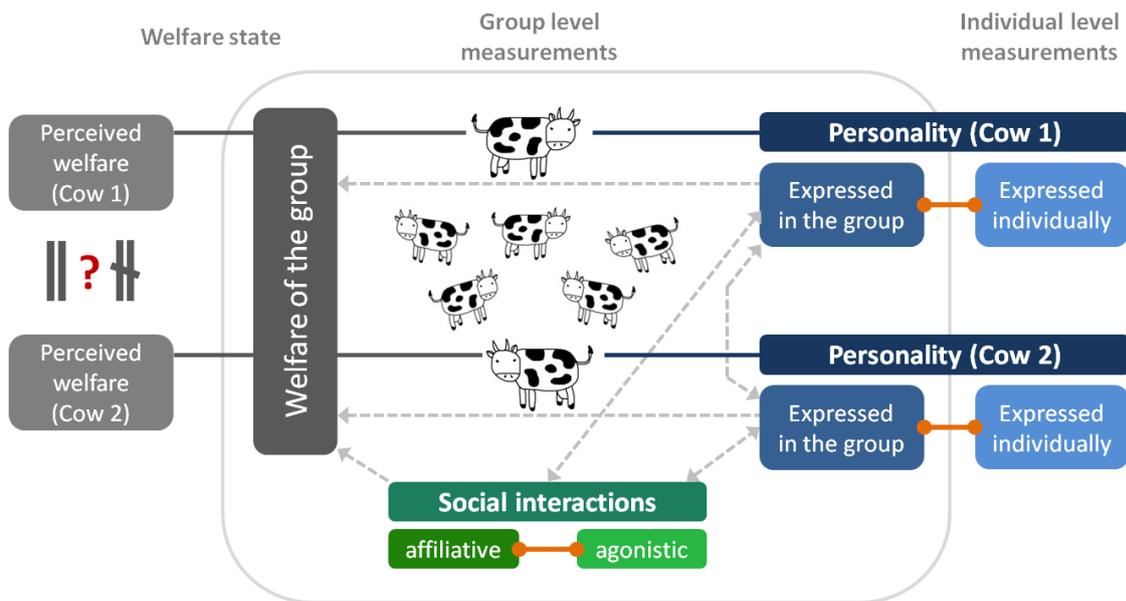
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# 1. General introduction

## 1.1. Motivation

The welfare of dairy cattle has gained increased scientific attention during the last decades due to ethical and societal concerns about modern husbandry systems (Rushen et al., 2008; von Keyserlingk and Weary, 2017). To assess animal welfare different aspects such as the health and biological functioning, affective states, and natural life of animals can be considered (Fraser, 2003, 2008). Moreover, the welfare of farm animals can be connected to their productivity (Blokhuis et al., 2003). Although welfare is a characteristic of individuals, traditionally it has been assessed at the farm- or group-level, and the focus has only recently been shifted to individual animals (Winckler, 2019). It is now recognized that there are differences between individuals within a group and individuals may significantly differ regarding their needs and welfare states (Richter and Hintze, 2019). In addition, technical advancement gave rise to “personalized” livestock farming, where individual-level data collection and management decisions are possible, and smart systems are of increasing importance in livestock husbandry (Rutten et al., 2013; Berckmans, 2014). In this context, behavioral observations in dairy cattle play an important role (Rushen et al., 2008): First, knowledge about the natural behavioral patterns of cows may serve as a basis to develop husbandry systems that fit their needs. Second, deviations from normal behavior can potentially be used as welfare indicators.

Adult lactating cows represent the majority of animals on a dairy farm and their welfare is of ethical and economic importance. However, the assessment of normal individual- and group-level behavior in lactating cows can be challenging due to various management routines associated with milk production (e.g., regrouping, milking, estrus and artificial insemination). Therefore, it is common to perform detailed behavioral observations in calves or heifers, and there is less knowledge about the behavioral variation in lactating cows. In this thesis I used a common framework for individual- and group-level analysis to better understand how the behavioral characteristics of individual animals and the behavior in the social group may influence welfare in lactating dairy cattle (**Figure 1**).



**Figure 1.** Possible relationships between welfare, social behavior, and personality on individual- and group-level, based on an example of two cows in a group. Topics illustrated with colors are considered in detail in this thesis. Gray areas and lines are a rough representation of assumed relevant relationships.

The existence of consistent individual differences in behavioral responses to stimuli in farm animals is a well-known fact among farmers (Fraser et al., 2017). In dairy cattle, this variation may also have practical relevance, as studies revealed an association between consistent individual behavioral differences (i.e., personality) and production traits (reviewed in Haskell et al., 2014). However, to practically measure and to consider personality in management decisions, it is important to take into account that the social environment might influence the expression of personality traits (Webster and Ward, 2011). Currently knowledge is limited about the personality traits adult dairy cows express within the every-day social environment, and it is unclear if these traits exhibit long-term temporal stability. Therefore, the first study in this thesis investigated the personality of lactating cows in individual and within-group situations, and the stability of personality traits was reassessed after a longer period of time.

In modern dairy husbandry systems groups are created and regularly changed according to the physiological status, milk production and nutritional needs of cows, and this can negatively affect their health and welfare (Chebel et al., 2016). However, despite the recognized importance of the social environment, knowledge about complex social interactions in groups of cattle is still limited (Boyland et al., 2016), partly due to the time consuming nature of data collection. Agonistic and affiliative interactions both contribute

to the social experience of cows, but in contrast to well-studied agonistic relationships, knowledge about the role of affiliative bonds is limited. Also, up to now no measure exists to integrate these behaviors into one practical descriptor of sociality. Thus, the second study complemented the individual-level personality assessment with a detailed analysis of social behavior to shed light on social behavioral variation in the every-day lives of dairy cows. Detailed video observation and social network analysis (SNA; see more details in section 1.4.5.) were applied for the comprehensive assessment of individual- and group-level affiliative and agonistic behavior.

In addition to furthering the understanding of social behavioral processes in dairy cow groups, there is a need for standardized and practical methods to enable the application of the new knowledge. As mentioned above, the time and labor intensive nature of human observations is a main factor limiting the use of behavior as practical welfare marker. In this regard the automatic detection of agonistic interactions is especially relevant, because they may directly be associated with social stress (Proudfoot and Habing, 2015). Therefore, the third study in this thesis validated an electronic-feeder-based algorithm in multiple facilities to record social competition, and to automatically assess the dominance hierarchy within lactating dairy cow groups.

The following sections provide a detailed introduction to the relevant topics of the thesis, specifically: concepts and methods related to individual and group welfare, personality, and social behavior, and methods for automatic social interaction detection in dairy cattle.

## 1.2. Welfare of the individual and welfare of the group

“...so far as the animals are concerned, it is not what we think or feel but what we do that counts” (Webster, 2016). What should we do to ensure the optimal welfare of farm animals in our care?

During the last decades, scientists and policy makers looked for answers to this question mainly along the framework of the *Five Freedoms* (Webster, 1994). This comprehensive outcome-based framework has widely been used as a basis for welfare assessment and livestock welfare management (Main et al., 2003; Welfare Quality, 2009). However, in recent years, theoretical advancements and an increased scientific understanding of biological processes resulted in a refinement of animal welfare thinking (Webster, 2016). Complementary approaches, such as the *Five Domains Model* (Mellor, 2017) and the *Quality of Life* concept (Mellor, 2016) are increasingly considered. The *Five Domains Model* includes the affective experiences of animals and their resulting mental states into the concept of animal welfare. The *Quality of Life* concept focuses on the balance between positive and negative experiences and aims at providing at least a “life worth living” or a “good life” for animals. Therefore, in contrast to early work focusing almost exclusively on avoiding negative welfare states (Mellor, 2012), it is getting clear that for ensuring optimal welfare not merely suffering should be minimized, but the positive experiences of farm animals (i.e., positive welfare) are also of high importance (Boissy et al., 2007; Yeates and Main, 2008; Mellor, 2015; Mellor and Beausoleil, 2015; Webb et al., 2019).

When looking for ways to provide a “good life” for a social species, such as dairy cattle, an important aspect is the relationship between the welfare of the group and the welfare of individual group members (Ohl and Putman, 2014). Current protocols, such as the Welfare Quality Protocol (Welfare Quality, 2009), usually assess welfare on the farm- or group-level (Andreasen et al., 2014; Winckler, 2019). However, it is not clear if the requirements for each individual within a group to achieve a “life worth living” are the same (Richter and Hintze, 2019). For example, it is known that individual cows show temporally and contextually stable differences in their reaction to environmental or physiological challenges (i.e., personality; see section 1.3.), which can have consequences for their welfare (Koolhaas and Van Reenen, 2016; Finkemeier et al., 2018). In addition, within the

every-day social environment of cows, the way an individual reacts to a challenge potentially influences the reactions of its group companions. Therefore, the personalities of cows and their interplay in the social group might be associated with differences in the perceived welfare status of individuals, even when they are exposed to the same environmental conditions (Ohl and Putman, 2014). Improving our knowledge about how individual behavior influences the welfare of other group members (Dunbar and Shultz, 2010) is necessary, if we want to provide cows with the freedom to perform normal behavior without letting this behavior to compromise the welfare of other individuals (Webster, 2016).

In humans, a positive social environment contributes to perceived security and has beneficial effects on health (Hennessy et al., 2009). In dairy cattle, the connection between the social environment and welfare has gained increased attention during the last few decades (Jóhannesson and Sørensen, 2000; Napolitano et al., 2009; Proudfoot et al., 2012). Long-term familiarity is related to social preferences in a group (Gutmann et al., 2015) and housing calves in groups increased their feed intake (Costa et al., 2015), or learning abilities (Gaillard et al., 2014). Moreover, many approaches discussed for the assessment of positive welfare are associated with the social environment (Boissy et al., 2007; Mellor, 2015). Specifically, affiliative bonds may be associated with positive emotions (Mellor, 2012), or the presence of familiar individuals can provide social support in stressful situations (Rault, 2012; Marino and Allen, 2017). However, the social behavior of dairy cattle is often compromised in modern farming systems, with limited opportunities for choosing social partners and maintaining meaningful bonds. Apart from a negative effect on welfare, the social environment can also be associated with increased stress, which could lead to higher disease risk (Proudfoot and Habing, 2015). Furthermore, the emotional states of individuals might be synchronized through emotional contagion (Špinko, 2012; Baciadonna et al., 2018), which may enhance or decrease the welfare of all individuals within a group. Finally, individuals within a group might also invest in the welfare of others: such prosocial behaviors (Rault, 2019) may improve the performance of the group as a whole and therefore the welfare of the given individual (Ohl and Putman, 2014). In summary, the association between the social environment and the individual- or group-level welfare of cattle is far from evident. Therefore, considering individual- and

group-level behavioral processes in a common framework is necessary to make practical suggestions for the improvement of welfare in dairy cattle groups.

### 1.3. Personality in cattle

In this section, I briefly introduce the definition and concept of animal personality, along with its proximate as well as ultimate causes. In addition, the section provides an overview of the measurement methods and the practical relevance of personality assessment in cattle.

#### 1.3.1. Definition and structure

The theoretical and historical basis of animal personality comes from human psychological research, where personality, i.e., the consistent behavioral variation of individuals, is mainly interpreted and categorized according to the *Five Factor Model* (Goldberg, 1990). This commonly used framework pictures personality along five dimensions and uses the following personality traits: extraversion, agreeableness, conscientiousness, neuroticism, and openness. Similar to the model used in human psychology, Réale et al. (2007) suggested a five-trait model as a framework to study personality in animals. The model considers the personality traits exploration, activity, boldness, sociability, and aggressiveness. This shows similarities to the human personality framework, as exploration resembles openness, aggressiveness could be paired with agreeableness, sociability with extraversion, and combining activity and boldness seem to resemble the human neuroticism factor (Finkemeier et al., 2018). In this context, dominance is also discussed as a possible sixth personality trait in animals, characterized by boldness, physical aggression and fearfulness (Gosling and John, 1999; Finkemeier et al., 2018). However, as Koski (2014) pointed out, some of these five personality traits might be found across species but they should not be treated as exclusive.

In animals, it was considered unscientific and anthropomorphic to refer to personality for decades (Goodall, 1998), even though scientific evidence supports the existence of consistent individual behavioral variation (Bell et al., 2009). This is probably one reason why animal behavioral studies describe personality with several different terms, such as temperament, coping style, or behavioral syndromes (for a detailed description of terminology see MacKay and Haskell, 2015; Finkemeier et al., 2018). During the last years, multiple studies suggested to apply the term personality in animals to refer to “a particular aspect of an individual’s behavioral repertoire that can be quantified and that

shows between-individual variation and within-individual consistency” (Carter et al., 2013). Throughout this thesis I also use this definition.

In cattle, the consistent behavioral characteristics of specific animals were traditionally categorized along one dimension (i.e., temperament), which captures the handling relevant between-individual variation, like docility, handling temperament, or milking temperament. With the advancement of knowledge about animal behavior and physiology, studying the multidimensional nature of behavioral variation also gained traction (e.g., Graunke et al., 2013). In cattle existing work found multiple traits to describe behavioral variation, and they have been connected with personality traits such as boldness, exploration, activity, fearfulness, sociability, and neuroticism (e.g., Van Reenen et al., 2004; Müller and Schrader, 2005; Gibbons et al., 2010; Graunke et al., 2013; Lecorps et al., 2018a,b). Therefore, it is likely that multiple personality traits exist in dairy cattle. However, there is no consensus yet which traits these are, and whether their relevance shows consistency throughout ontogeny. One reason for this is that many studies perform measurements in calves, repeat these within a short period of time (i.e., weeks) and less is known about the personality of adult cows.

### **1.3.2. Causation, function, ontogeny, and evolution**

Personality may be better understood in detail when considering it along the four fundamental questions, formulated by Nico Tinbergen (Tinbergen, 1963) to facilitate the understanding of any behavior (Budaev and Brown, 2011; Bateson and Laland, 2013):

*Causation: What is the underlying mechanism of personality?*

Personality is influenced by genetical factors and the manifestation of personality traits has a neurological basis (MacKay, 2018). In humans, recent work shed light on its proximal neurological mechanisms and how different brain structures are related to aspects of personality (Bjørnebekk et al., 2013; Kennis et al., 2013). In cattle, genetic studies looked into the background of the behavioral reaction to challenging situations and identified genomic regions related to behavioral phenotypes (Gutiérrez-Gil et al., 2008; Friedrich et al., 2016). Moreover, despite the environmental factors that are expected to affect behavior, reasonable heritability estimates (ranging between low and medium) were

described for temperament in dairy and beef cattle (Haskell et al., 2014; Friedrich et al., 2015; Koolhaas and Van Reenen, 2016; Stephansen et al., 2018). However, due to the complexity of behavioral traits and their polygenic nature, knowledge is still limited about the genotype – phenotype interactions (Friedrich et al., 2015). Also, it is not entirely clear how and which genetical, neurological, and metabolic factors play a role in forming individual behavioral variation in cattle.

*Function: What is the adaptive value of personality?*

Personality is proposed to be an adaptation to differences in state variables of individual animals (Sih et al., 2015), such as morphology, physiological condition, available information, and environment (Wolf and Weissing, 2010), with a positive feedback loop, where behavioral differences may stabilize the initial differences in state (Sih and Bell, 2008). The advantages of consistent individual behavioral differences within a population may be twofold: First, consistency of behavior means that the reactions of an animal are predictable to some degree. This may be especially advantageous in social interactions because responsive individuals can take into account the behavioral history of their social partners (Wolf et al., 2011). Second, diversity of the behavioral reactions within a group enables the presence of phenotypes which are not possible in single individuals (e.g., being bold and shy at the same time). When the behavior is consistent within individuals, variation between individuals in a group may produce benefits for all group members (Nonacs and Kapheim, 2008; Farine et al., 2015). In addition, stable personalities within a group may provide higher chance that some individuals can successfully cope with changing environmental conditions (Koolhaas et al., 1999).

*Ontogeny: How does personality develop?*

By definition, personality traits show consistency over time within individuals (cf., section 1.3.1.). However, this stability might be temporary and personality may change between life stages, when the body undergoes morphological and physiological reorganization, such as early in life or during sexual maturation (Stamps and Groothuis, 2010). Recent theoretical and empirical advancements indicate that personality can be less stable than often thought (Groothuis and Trillmich, 2011), and there might also be individual differences in the plasticity of personality traits over ontogenesis (Stamps and Biro, 2016).

However, personality can be stable through ontogeny even in species with complex life cycles, like metamorphosis (Koenig and Ousterhout, 2018). In cattle, many studies repeated personality tests within relatively short time periods (cf., section 1.3.1.), and thus not relevant in terms of ontogenesis. Some work also assessed the stability of personality traits over a longer period of time. Results suggest that personality of calves may be more plastic during the first weeks of life and become stable afterwards (e.g., Van Reenen et al., 2005; Haskell et al., 2012).

#### *Evolution: How did personality evolve?*

In wild animals, according to the theory of Wolf et al. (2011), the existence of personality (i.e., limited behavioral plasticity of individuals) may be surprising from an adaptive perspective. However, within a population predictability of behavior can be beneficial. From an evolutionary point of view, the presence of different behavioral strategies (i.e., individuals within a group consistently differing in the reaction to a challenge) may be advantageous because it provides the group with a level of flexibility and facilitates adaptation when environmental conditions change. The commonly used pace-of-life syndrome theory (Réale et al., 2010) suggests that personality co-evolved with life-history, morphological, and physiological traits, but a recent meta-analysis (Royauté et al., 2018) raised questions about the general applicability of this hypothesis. Besides the above mentioned theories, it is also possible that personality is more of an “accidental” leftover random variation in individual behavior, because there was no strong selection pressure for a particular personality type (MacKay, 2018). In the wild ancestors of cattle, evolution could have influenced the consistent individual behavioral differences, whereas after domestication most of the genetic change came from selective breeding for production traits. It is still unclear how personality is influenced through breeding e.g., due to genetic correlations between production traits and behavioral traits (Oltenacu and Broom, 2010). However, several studies indicated that consistent individual variation is present in farm animals in spite of domestication and breeding (Koolhaas and Van Reenen, 2016; Finkemeier et al., 2018) and it also has practical relevance.

### **1.3.3. Practical relevance**

During the last years interest in the detailed measurement of personality traits in cattle is growing, because their practical relevance has been recognized. For instance, in beef cattle a relationship was found between temperament measurements (cf., section 1.3.4.) and average daily weight gain (Müller and von Keyserlingk, 2006), feed efficiency (Cafe et al., 2011), or meat quality (King et al., 2006). In dairy cattle less work has been done, but fearfulness was found to be correlated with lower milk yield (Breuer et al., 2000; Hemsworth et al., 2000), and a relationship between behavioral reactions to milking, personality tests, and milk production was also suggested (Sutherland et al., 2012; Sutherland and Dowling, 2014; Hedlund and Løvlie, 2015). Friedrich et al. (2016) found in the F<sub>2</sub> generation of a Charolais x German Holstein cross-breed population that genotypes associated with low levels of activity and exploratory behavior were related to higher milk yields, however the exact mechanism is not known. Furthermore, personality is associated with individual variability in feeding behavior in domesticated ruminants (reviewed by Neave et al., 2018a) and recent work with dairy calves found that active and exploratory individuals had a higher average daily weight gain (Neave et al., 2018b). Due to the possible effect on productivity and the associations between ease of handling and temperament, personality is increasingly considered as a breeding relevant trait. Handling temperament and milking temperament are included in breeding value calculations, but due to the lack of calculated economic values, temperament is usually not incorporated in selection indices (Haskell et al., 2014). Existing work implies that including behavioral traits, more explicit than temperament, in breeding programs might be beneficial (Adamczyk et al., 2013; Haskell et al., 2014). However, the lack of standardized robust behavioral measures on a large number of animals (see section 1.3.5.) hampers the assessment of the relevance of specific personality traits for breeding.

Personality likely has practical implications for dairy cattle welfare on the individual- level. In humans, research with twin pairs showed that the subjective well-being and personality of individuals is linked by common genes (Weiss et al., 2008). Although no such evidence exists at the moment for cattle, a recent study showed that fearful calves made more pessimistic judgements in a cognitive bias test (Lecorps et al., 2018b). In addition, a relationship between personality, stress response and immune function of dairy cattle is suggested (Koolhaas and Van Reenen, 2016). If specific personality types are less able to

cope (Koolhaas et al., 1999) with environmental challenges (e.g., because they are more fearful) it may lead to chronic stress, deprived immune function (Hopster et al., 1998) and compromised health.

The personality of individuals might also have practical relevance for the functioning and social structure of the group they live in (Herbert-Read, 2017; Jolles et al., 2017). In wild animals the phenotypic composition of a group (in terms of personalities of group members) might affect group-level outcomes, thus impacting individual fitness (Farine et al., 2015). Accordingly, the personality composition of a group may have direct relevance for individual-level and group-level welfare in dairy cattle. For instance, exploratory behavior or aggressiveness of specific individuals can influence the level of competition, or it may disturb the feeding and lying behaviors of others. In addition, the behavior of individuals within a group might be modulated by the availability of resources (according to hawk-dove games; Houston and McNamara, 1988). Furthermore, the social behavioral fine-structure of the group might also be influenced by personality through individual differences in social responsiveness or preferred interaction partners (Wolf and Krause, 2014).

Despite the clear practical relevance in dairy cattle, up to date very little is known about how personality is expressed within the social group (Webster and Ward, 2011). The social environment may have an influence on the behavior of individual cows through multiple mechanisms. For example, cattle are herd animals, they tend to synchronize their behavior within the group, therefore social conformity and social awareness may lead to individuals acting differently within the group than they would alone (Veissier et al., 1998). Also, social facilitation may be an important behavioral modifier, as substantial evidence exists in calves that social facilitation influences foraging behavior (Costa et al., 2015). In addition, the phenotypic composition and social behavioral structure of a group may also influence whether and how the above mentioned processes take place.

#### **1.3.4. Personality measurement and analysis**

Temperament tests were performed in cattle already several decades ago for the practical measurement of individual behavioral variation. Although these tests enable the practical classification of animals, as mentioned in section 1.3.1., temperament in cattle is often treated at a one-dimensional scale and captures only the behavioral differences which are directly handling relevant. In beef cattle most common tests are the chute test (also called as crush test), flight speed, and docility test; whereas in dairy cattle mainly the response to milking or handling was measured (see Haskell et al., 2014 for a review and description of tests).

Contrary to temperament tests, experimental circumstances enable the use of a multi-trait multi-test approach, thus a more detailed description of cattle personality. The following fear tests (Forkman et al., 2007) are commonly used to measure the reaction to novelty and for the characterization of personality traits such as boldness, activity, and exploration (Réale et al., 2007):

- **Arena test/open field test:** Behavioral reaction to a novel environment and social isolation is measured. The cow is led to an unfamiliar test arena and stays there for several minutes.
- **Novel object test:** Performed combined with the arena test or as a single test. Behavioral reaction to an unknown object is measured in a test arena.
- **Novel human test:** Performed combined with the arena test or as a single test. Also called as voluntary human approach test. Behavioral reaction to the presence of an unknown human is measured in a test arena.

Similar tests are used to quantify behavioral differences between cows in a familiar environment:

- **Forced human approach test:** The flight distance of the cow is measured when a human approaches it at the feed bunk, walking alley or lying stalls (Waiblinger et al., 2006; Gibbons et al., 2009a).
- **Reaction to novel stimuli in a familiar environment,** such as presenting novel food, unknown human, novel object (Herskin et al., 2004), or flashing light and water spray (Gibbons et al., 2009a).

Additional measurements are used for the quantification of traits which are relevant in a group context (i.e., sociability and aggressiveness):

- Runway test: It is measured how much time it takes for an isolated cow to go back to its peer group (Gibbons et al., 2010; Lecorps et al., 2018b).
- Aggressiveness is usually measured with quantifying the amount of agonistic interactions initiated by a cow, for example at the feed bunk directly after the provision of fresh feed (DeVries et al., 2004; Gibbons et al., 2009b).

Personality traits are commonly derived from several behavioral parameters measured in a single test or in multiple tests. For the statistical analysis, principal component analysis (PCA) is often used in human and animal personality research (Budaev, 2010). Briefly, PCA is a multivariate dimension reduction method, which condenses correlated measures into principal components. The loadings of each principal component show the size of the correlation between the original parameter and the particular principal component. Different behavioral parameters measured in personality tests often reflect the same underlying personality trait. Therefore, to reduce the number of parameters used and to increase their reliability PCA is an ideal tool. However, if many parameters are recorded, high sample size is required for robust analysis, with roughly five times as many animals as parameters used in the PCA (Budaev, 2010). The gained principal components may be interpreted as personality traits based on the loadings of the behavioral parameters. Thereafter the scores of individuals for each principal component can be used as a measure of that specific personality trait. However, the interpretation of components can be subjective, making it difficult to compare trait scores between studies.

### **1.3.5. Practical personality assessment**

To facilitate the routine application of personality in animal welfare and breeding, it is required to be able to collect relevant personality data on a large number of animals in a practical manner. Several studies applied standardized behavioral tests and analysis methods in dairy cattle and obtained multiple personality traits (e.g., de Passillé et al., 1995; Müller and Schrader, 2005b; Van Reenen et al., 2005; Graunke et al., 2013; Lecorps et al., 2018b; Neave et al., 2018b). However, in most studies calves were tested and the behavioral parameters were almost exclusively recorded in individual test situations.

Therefore the results may not accurately represent the variation in behavioral reactions which is manifested in the every-day social environment of adult dairy cows. Existing work found a relationship between primary behavioral measures in the home pen, such as lying behavior or sociability, and individual personality test results (Gibbons et al., 2010; MacKay et al., 2014; Lecorps et al., 2018a). However, contrary to the variety of widely used individual tests, currently no feasible test is known which could be applied within the social group of dairy cows. A test measuring the reaction to novelty within the home pen might provide a reliable measure of personality traits, so a few studies (Herskin et al., 2004; Gibbons et al., 2009a) used this approach, but here animals were tested in the home environment individually. Therefore, it is not known whether such a test produces reliable results when the entire social group is present. For a test to be potentially used under commercial farm settings it has to be easily applicable and results should be assessable without laborious data analysis. Hence, a simple novel object test, where the contact or closeness to the novel object is measured as a parameter, might be a good candidate for a practicable test, if it correlates with comparable measures from individual tests.

## 1.4. Social behavior in cattle

This section provides an overview about the challenges associated with living in a social group on commercial dairy farms, briefly reviews the current knowledge about agonistic and affiliative behavior of cows, and introduces the analysis methods which were used to assess social behavior in this thesis.

### 1.4.1. Living in a group

The importance of social relationships is long known in humans. In fact, it was shown to be one of the main determinants of longevity (Yang et al., 2016). The relevance of social behavior is also recognized in farm animals: *“Advancing our knowledge of social behavior is of fundamental importance for maximizing the productivity and welfare of animals held in captivity”* (Krause et al., 2015). However, it is still unclear what living in a group means for dairy cows in terms of social behavioral structure and the social experience of individuals.

Free-roaming animals belong to a group when the benefits exceed the costs of group membership and may join or leave the group according to the cost-benefit ratio, thus regulating group size (Estevez et al., 2007). In cattle, being the member of a group has benefits such as decreased predation risk, social thermoregulation, or social learning, but it comes with costs like competition for resources. Descriptions of feral cattle living under natural circumstances indicate that they form groups of around 20 individuals (however, groups sometimes merge to much larger herds) consisting mostly females and sub-adult males; whereas males usually live separately in smaller groups (Bouissou et al., 2001). Contrary to feral cattle, dairy cows in confined environments under commercial conditions do not have the possibility to establish a group of a given size based on associated costs and benefits. Moreover, their social life is strongly influenced by the management of the production system. Dairy calves face their first social challenge shortly after birth when they are separated from the dam and housed individually during the first weeks of their lives. The formation of social bonds only starts after weaning, when calves are introduced to a peer group (Flower and Weary, 2003). Social isolation during this early period can have significant negative effects and recent research emphasizes the benefits of rearing calves in social groups (Gaillard et al., 2014; Costa et al., 2016). Dairy heifers

face another challenge around the time of the first calving: after the stress of giving birth, they are separated from the offspring, introduced to a new social group, and have to adapt to new management practices such as milking. In addition, cows are regrouped several times during lactation according to milk production and nutritional needs. Consequently, they regularly experience the breaking of existing social bonds and have to establish new ones, which can negatively affect their behavior and welfare (von Keyserlingk et al., 2008).

Social relationships in dairy cattle have mostly been studied based on dyadic interactions, which can be divided into two broad categories such as socio-positive (affiliative) and socio-negative (agonistic) behavior (Bouissou et al., 2001; Rault, 2019). Up to date, most work analyzed these behaviors separately. As a result, knowledge about the complete social behavioral structure of dairy cow groups is still limited. One reason for this is the highly time and labor intensive nature of traditional behavioral observations and that complex analysis methods, like SNA (Wey et al., 2008; Whitehead, 2008), only recently gained popularity in farm animal behavioral research (Makagon et al., 2012). In the following I will provide a short introduction to the main social interaction types and analysis methods applied in cattle social behavior studies.

#### **1.4.2. Agonistic behavior**

Under modern housing conditions, agonistic interactions mostly occur when cows are overstocked and they have to compete for resources, and also when they reestablish the social hierarchy after group composition changes. For example, after regrouping an increase in agonistic interaction frequency can be observed (Kondo and Hurnik, 1990; von Keyserlingk et al., 2008), but otherwise the level of aggression is reported to be rather low (Bouissou, 1980). Agonistic interactions can be classified as threats without physical contact (head swing or lateral display) which usually result in the retreat of the threatened animal, or in a response threat, and eventually in fighting (Bouissou et al., 2001). In addition, head butt or displacement is a common interaction that is usually used by the actor cow to gain control over a resource. Hence, such interactions are commonly observed at the feed bunk or at other resources such as the lying stalls, or even at a mechanical brush (Val-Laillet et al., 2008b).

Live or video observations of agonistic behavior in dairy cattle groups were performed already several decades ago (Schein and Fohrman, 1955). However, most studies considering dairy cows in a barn recorded agonistic behavior for relatively short daily sampling periods (but see Val-Laillet et al., 2009 as an example for continuous observation over days). Moreover, often only the feed bunk area was observed, especially during the first few hours after providing fresh feed, since agonistic interactions are frequent during this period (DeVries et al., 2004; Gibbons et al., 2009b). Analyses of agonistic interactions resulted in a considerable amount of evidence that the social competition in cattle groups is regulated through dominance relationships (Bouissou, 1980; Kondo and Hurnik, 1990; Wierenga, 1990; Val-Laillet et al., 2008a). Dominance can be analyzed on different levels, such as 1) the group-level, where the linearity and transitivity of the hierarchy is studied, 2) the dyadic-level where the symmetry of the relationship is considered, and 3) the individual-level where the dominance rank of a particular animal is in focus (Langbein and Puppe, 2004). Regarding small dairy cow groups (i.e., 8-12 cows) housed in free-stall barns, analysis of dominance based on detailed video observations indicated that the social hierarchy is not entirely linear and many circular triads are present (Val-Laillet et al., 2008a, 2009). An established dominance hierarchy serves to regulate the social structure and to decrease the number of agonistic interactions in the group. However, regrouping or overstocking cows is relatively common on modern dairy farms, and this practice can be a source of increased agonistic interactions even when the dominance status of cows shows stability. Moreover, particularly aggressive individuals may disturb the feeding or resting behavior of others within the group even when it is stable.

Up to date, it is not exactly known what role frequent agonistic interactions and dominance ranks play in the health and welfare of cows. An association between dominance and individual stress level in group-living vertebrates is suggested in the literature (Otten et al., 1999; Creel et al., 2013; Miranda-de la Lama et al., 2013), but this connection is not straightforward and probably depends on the species and how dominance is acquired and maintained in the group. Knowledge about the fine structure of agonistic behavior and its changes in the dairy cattle groups is still limited, therefore the connection between the welfare of group members and the changes in social behavioral patterns is understudied.

### 1.4.3. Affiliative behavior

Although extensive attention has been given to agonistic behavior, much less scientific work has been done on affiliative behavior in farm animals (Rault, 2019). In cattle, early work investigated dyadic relationships based on long term live observation of spatial and social behavior and provided evidence of long lasting affiliative bonds or friendships (Reinhardt and Reinhardt, 1980; Wasilewski, 2003). Moreover, it was suggested that reciprocal affiliative bonds can positively influence the health and welfare of animals under intensive farming conditions (“Regelkreismodel”; Wasilewski, 2003). Despite early results, the role of peers and socio-positive interactions in confined environments gained more attention in recent years only (Val-Laillet et al., 2009; Tresoldi et al., 2015; Boyland et al., 2016; Rault, 2019). Research showed that dairy cows maintain valuable social relationships and prefer social partners they are familiar with (Gutmann et al., 2015). Furthermore, the removal of conspecifics and thus the breaking of social bonds might impact feeding behavior and immune function in cows (Walker et al., 2015).

For the assessment of affiliative bonds in cattle either grooming interactions (i.e., social licking) or associations based on distances or common activity (i.e., nearest neighbors, lying together, or feeding together) are used. Licking behavior is often performed after solicitation and it is mostly directed to the head or neck of the receiver, since these body parts are inaccessible for the receiver itself (Bouissou et al., 2001; Val-Laillet et al., 2009). Up to date, the different functions and regulating mechanisms of grooming behavior in cattle groups are not entirely clear. It is suggested that licking plays a role in maintaining coat hygiene (Boissy et al., 2007) and forming social bonds (Sato et al., 1991; Dunbar and Shultz, 2010). In addition, social licking might have a calming effect and serve to reduce social tension (Laister et al., 2011), however Val-Laillet et al. (2009) found a decreasing total time of grooming with increasing social pressure. Furthermore, housing conditions (i.e., free stall or pasture) can also have a significant impact on the frequency of grooming, with indoor housing being associated with more frequent affiliative interactions (Tresoldi et al., 2015). Spatial proximity was also found to be associated with increased grooming within pairs of cows (Tresoldi et al., 2015; Boyland et al., 2016) and grooming was observed more often in dyads who were frequent neighbors at feeding (Val-Laillet et al., 2009).

Previous work mainly focused on the function of grooming or licking and considered dyadic affiliative relationships. Therefore knowledge about the group-level structure of affiliative interactions and on the relationship between the agonistic and affiliative behavioral structure within dairy cow groups is still limited. However, advancements in analysis methods for investigating social behavior on a group-level may help to overcome some of the difficulties (e.g., lack of common framework to study agonistic and affiliative behavior) and enable to gain more insights on the social experience of cows.

#### **1.4.4. Combining affiliative and agonistic behavior**

In cattle, a few studies investigated the association between the dominance hierarchy and the expression of grooming behavior and yielded inconsistent results. Studies either found that mostly subordinate cows groom dominant ones (Sato et al., 1993), grooming is directed down the hierarchy (Šárová et al., 2016), or no association between dominance and grooming (Sato et al., 1991; Tresoldi et al., 2015). In addition, it is suggested that grooming may serve as a mechanism to reduce social tension (Nakanishi et al., 1993; Šárová et al., 2016), but evidence also exists to the contrary (Val-Laillet et al., 2009). These inconsistencies might be due to behavioral differences between beef and dairy cattle and due to methodological differences between studies. Moreover, these studies considered dominance, which is a measure derived from group-level agonistic interaction patterns, whereas to represent affiliative relationships grooming interactions were used without further considering their group-level structure.

#### **1.4.5. Social indices and social network analysis (SNA)**

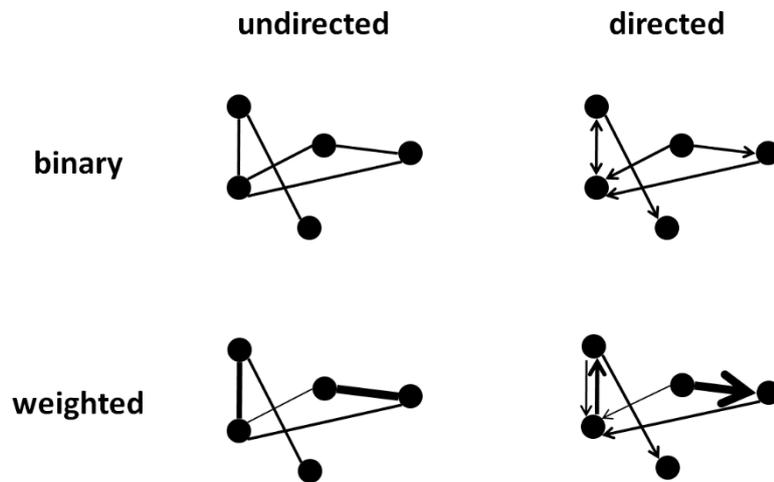
As outlined in the previous sections, existing work mostly focused on either affiliative or agonistic relationships when assessing social behavior in cattle groups. For agonistic behavior the most commonly used analysis is the calculation of dominance, either on individual-, dyadic-, or group-level. In cattle several different indices (e.g., Lamprecht Index, Galindo-Broom Index, Mendl Index, Kondo-Hurnik Index) were used to assess the dominance rank of individuals in a group, but no consensus on the best approach exists (Langbein and Puppe, 2004; Val-Laillet et al., 2008a). When studying dominance in wild animals, behavioral ecologists use a range of different dominance measures which are less often applied in farm animal behavioral research. Such measures are for example the

David's score (David, 1987), which relies on the agonistic interaction matrix, or the Elo-rating (Albers and De Vries, 2001), where the temporal sequence of agonistic interactions is also considered. Recent work comparing the performance of dominance calculation methods based on simulations found that the David's score produces reliable results and that approximately 10-20 times as many interactions as animals in the group are already sufficient for hierarchy estimations (Neumann et al., 2018; Sánchez-Tójar et al., 2018). Moreover, the normalized David's score is independent from group size and it can be calculated based on data sets where dyadic dominance relationships are corrected for chance (see De Vries et al., 2006). Therefore in this thesis the normalized David's score was used for measuring dominance.

Contrary to dominance, no generally used index exists for the description of affiliative relationships on dyadic or group-level. Due to the relatively low frequency of affiliative interactions and observation difficulties, physical closeness is often used instead of actual interactions to infer affiliative relationships within a group (Farine and Whitehead, 2015). However, in a confined environment animals only have limited possibility to keep distance from each other, and closeness in the pen may also be biased by common spatial preferences. Therefore, relying only on closeness for the assessment of affiliative relationships in indoor-housed cattle might produce results different from those based on observed affiliative interactions.

In dairy cattle affiliative and agonistic interactions have not yet been considered using a common analysis framework. SNA is one tool which can be used to better understand the complexity and interplay of different social interactions beyond dyadic relationships. SNA enables the detailed analysis of social behavior on multiple levels (i.e., individual-, dyadic-, or group-level) and it is increasingly used in applied ethology (Makagon et al., 2012; Kleinhappel et al., 2016). For a detailed introduction to SNA in animal groups, readers are referred to a recent paper and book (Farine and Whitehead, 2015; Krause et al., 2015) on the topic. Here I provide a brief summary of the method. In a social network, nodes represent individuals within a group and edges represent some kind of relationship between them. An edge between two nodes can be binary (i.e., the nodes are either connected or not) or weighted, when the strength of a relationship is also considered. In addition, edges may be directed, in which case one node is the actor and the edge points

to the recipient. **Figure 2** presents an example for each of these network types using a group of five individuals.



**Figure 2.** Exemplary social networks. Black points represent the individuals (nodes) and lines represent the relationships (edges) between them. In weighted networks the width of the line reflects the strength of a relationship. In directed networks arrows point from the actor to the receiver.

Typically, directed networks are used when social interactions are observed in a group and undirected networks are created with association (e.g., closeness or avoidance) data. In a barn environment usually all members of a group can be observed at each sampling event, individuals have limited opportunities to keep distance from others, and social interactions occur regularly. Therefore, under these conditions the use of weighted and directed networks is practicable in order to avoid the occurrence of almost completely saturated undirected networks with binary edges which provide little information on the fine structure of social behavior. Using SNA, several node-level and network-level measures can be calculated to better understand complex social behavioral patterns. The most common measures are shown in **Table 1**.

**Table 1.** Common social network metrics and their definitions

| <b>Node – level measures</b>    |  |
|---------------------------------|--|
| Degree                          | Number of edges connected to a node;<br>IN-Degree and OUT-Degree in directed networks                          |
| Strength                        | Weighted degree; sum of edge weights connected to a node;<br>IN-Strength and OUT-Strength in directed networks |
| Betweenness                     | Number of shortest paths going through a node  |
| Eigenvector centrality          | Sum of the degree of a node's neighbors  |
| <b>Network – level measures</b> |  |
| Density                         | Percentage of existing edges in a network  |
| Assortativity                   | Shows if nodes with similar characteristics are more or less connected than expected                           |
| Transitivity                    | Clustering coefficient; tendency of groups of nodes to be interconnected                                       |
| Dyadic census                   | Number of mutual, asymmetric and null relationships in directed networks                                       |

SNA is an advanced analysis method, but it requires extensive data collection on affiliative or agonistic relationships. In addition, ensuring the meaningfulness of input data is a critical step, since spurious data can have considerable consequences on the results and conclusions drawn. However, currently no sampling guidelines exist for collecting data on affiliative or agonistic interactions in dairy cattle groups in a free-stall barn. Up to date, studies using SNA based on observed social interactions are sparse in cattle (but see Šárová et al., 2016 for an example in beef cattle). Applying SNA for the comprehensive analysis of observed affiliative and agonistic interactions in dairy cow groups appears to be a promising way to integrate these behaviors into one measure of social experience.

## **1.5. Automatic recording of social behavior**

To facilitate the practical use of social behavior, not only advancing the scientific understanding of behavioral processes, but also developing efficient and reliable data collection methods is necessary. In this section, I briefly introduce the traditional and novel approaches to collect data on cattle social behavior. In addition, I highlight the challenges associated with the validation and routine use of automatic data collection methods.

### **1.5.1. Data collection on social behavior**

One main issue hindering the analysis and understanding of social behavioral structures in indoor-housed dairy cattle is that traditional live or video observations are time consuming and labor intensive. To overcome this problem, many studies limit the sampling of social interactions in space or time. Often only specific areas such as the feed bunk are observed, or only those time periods are considered when interactions are known to be frequent, for instance after fresh feed delivery (e.g., Sato et al., 1991; DeVries et al., 2004; Tresoldi et al., 2015). However, no robust guideline is available for indoor-housed dairy cows regarding the sampling of affiliative or agonistic interactions, and it is not known how limited sampling impacts the conclusions drawn.

To assess affiliative relationships physical closeness is often used instead of the observation of social interactions, as mentioned in the previous section. One reason for this is the increasing availability of different technical solutions for the automatic measurement of distances between cows in a group. For example, Boyland et al. (2013) validated proximity loggers, and they found a correlation between logger-based association and actual grooming interactions in dynamic dairy cow groups (Boyland et al., 2016). Others used high resolution location data to measure the association between cows (e.g., Gyax et al., 2010; Koene and Ipema, 2014; Chen et al., 2015), but parameter settings to define associations were often chosen arbitrarily. In this context, it is unknown to which extent location based affiliation data describes the same social behavioral patterns as observed social interactions. Agonistic interactions are frequent in areas where the competition for resources is high, therefore these zones were recently considered for the automatic detection of displacements. The waiting area of an

automatic milking station was used to test the suitability of video image analysis to detect agonistic interactions (Guzhva et al., 2016, 2018). In addition, an electronic feeder system that originally serves to measure the feed and water intake of cows (Chapinal et al., 2007) has been validated to detect replacements at feed or water bins (Huzzey et al., 2014; McDonald et al., 2019). Although these studies provided the first steps for detecting agonistic interactions, they have been validated on the individual-level using focal cows. Up to date it has not been studied in detail if such methods can provide a reliable picture of the complete agonistic behavioral structure in groups of cows.

### **1.5.2. Validation and use of automatic methods**

With the increased availability of precision livestock farming (Berckmans, 2014) and the advancement of digitalization in dairy barns it seems to be possible to routinely collect data on the social environment of cows using automatic methods. As described above, first attempts have been made and show promising results. However, no guideline exists for choosing parameter settings in different environments, and the general applicability of the automatic methods is also unclear, because most of them were only used once in a specific barn. Another issue is that due to laborious traditional data collection, the validation of automatic methods often relies on observations of focal animals or on limited sampling periods (e.g., Huzzey et al., 2014; Boyland et al., 2016). However, automatic methods collect continuous data and therefore potential systematic errors, overseen due to limited validation, may bias the results. In this work, the detailed data collection on affiliative and agonistic interactions using continuous video observation over multiple days in dairy cow groups presents a unique opportunity for the thorough validation of automatic data collection methods. In this regard, focusing on agonistic interactions is particularly important. Specifically, data on the total agonistic interactions in the group enables to assess if interactions detected at specific areas in the pen, such as the feed bunk, reliably represent the agonistic behavioral structure on the group-level. The automatic detection of social competition could enable the detailed investigation of the temporal stability of dominance and aggressiveness as personality traits, and facilitate future work concerning the relationship between agonistic interactions and other factors such as stress, illness, or productivity.

## 1.6. Aims and hypotheses

The personalities of individual cows and the group-level patterns of affiliative and agonistic interactions are potentially interconnected. Moreover, these connections may influence the welfare of cows in ways which we currently have limited knowledge of. Therefore, in this thesis I analyzed the behavior of lactating dairy cows on the individual- and group-level to address three main objectives:

The goal of the **first study** was to investigate personality. Specifically, to assess if 1) personality traits, revealed by a number of classical individual tests, express stability over a longer period of time, and 2) personality shows consistency between individual and group contexts, measured by a developed practicable group test in the home pen. The study was based on the hypothesis that dairy cows exhibit multidimensional personality which is stable over six months, and show some behavioral consistency between individual and group contexts.

The goal of the **second study** was the comprehensive analysis of the social environment. Specifically, to 1) determine a suitable time scale for the analysis of affiliative and agonistic interactions, 2) investigate the relationship between the structure of these two behaviors and also combine them into one measure, and 3) assess the long-term temporal stability of individual social behavioral characteristics. Accordingly, the hypothesis was that multiple days of observation is necessary to gain a reliable picture of the agonistic and affiliative behavior in the group, dominance has some association with grooming patterns, and the social behavior of cows shows consistency over time.

The goal of the **third study** was to facilitate the automated assessment of agonistic behavior on the group-level. Therefore, an electronic-feeder-based algorithm was validated for detecting the dominance hierarchy in lactating dairy cow groups in different facilities. This validation was based on the hypothesis that 1) agonistic replacements at the feed bunk can be reliably captured using combined data from electronic feed and water bins, and 2) the detected interactions provide a good approximation of the dominance hierarchy obtained based on all observed agonistic interactions in the pen.

## 2. Experimental studies

### 2.1. Evaluating the temporal and situational consistency of personality traits in adult dairy cattle

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Borbala Foris designed and performed the experiment, analyzed the data, and wrote the manuscript with the support of and in agreement with her supervisor Dr. Nina Melzer and the co-authors of this manuscript.

## Evaluating the temporal and situational consistency of personality traits in adult dairy cattle

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### Abstract

Recent research suggests that personality, defined as consistent individual behavioral variation, in farm animals could be an important factor when considering their health, welfare, and productivity. However, behavioral tests are often performed individually and they might not reflect the behavioral differences manifested in every-day social environments. Furthermore, the contextual and longer-term temporal stability of personality traits have rarely been investigated in adult dairy cattle. In this study, we tested three groups of lactating Holstein cows (40 cows) using an individual arena test and a novel object test in groups to measure the contextual stability of behavior. Among the recorded individual test parameters, we used seven in the final analysis, which were determined by a systematic parameter reduction procedure. We found positive correlations between novel object contact duration in the group test and individual test parameters object contact duration ( $R_s = 0.361$ ,  $P = 0.026$ ) and movement duration ( $R_s = 0.336$ ,  $P = 0.039$ ). Both tests were repeated 6 months later to investigate their temporal stability whereby four individual test parameters were repeatable. There was no consistency in the group test results for 25 cows tested twice, possibly due to group composition changes. Furthermore, based on the seven individual test parameters, two personality traits (activity/exploration and boldness) were identified by principal component analysis. We found a positive association between the first and second tests for activity/exploration ( $R_s = 0.334$ ,  $P = 0.058$ ) and for boldness ( $R_s = 0.491$ ,  $P = 0.004$ ). Our results support the multidimensional nature of personality in adult dairy cattle and they

indicate a link between behavior in individual and within-group situations. The lack of stability according to the group test results implies that group companions might have a stronger influence on individual behavior than expected. We suggest repeating the within-group behavioral measurements to study the relationship between the social environment and the manifestation of personality traits in every-day situations.

## Introduction

Recently, there has been a growth in interest in the connections between personality, health, welfare, and productivity in farm animals [1,2]. It has been suggested that different personalities may vary in terms of their disease susceptibility [3], physiological response to stress [4,5] and production traits [6-11]. Furthermore, considering personality in the context of animal breeding seems to be a promising approach for improving the robustness and welfare of farm animals [1,12]. Personality is applied as a term in many species to refer to individual behavioral variation that is stable across time and context [13,14]. However, the term temperament is often used when referring to farm animals, probably to avoid anthropomorphism [15-17]. In the present study, we use the term personality trait to refer to “a particular aspect of an individual’s behavioral repertoire that can be quantified and that shows between-individual variation and within-individual consistency” [18]. The framework proposed by Réale et al. [19] generally considers five personality traits in animals: activity, exploration, boldness, sociability and aggressiveness (similar to the “Big-Five factor model” used in humans [20]). There is still debate regarding whether these personality traits are exclusive and if they can be assessed in all species [21].

Several studies have assessed the multidimensional character of personality in calves (e.g. [22-24]) and multiple personality traits have been reported. However, personality traits and their stability might change throughout ontogenesis [25,26] and the contextual and longer-term temporal stability of personality traits in adult lactating cows has rarely been investigated (but see [27,28]). Personality is commonly assessed using individual tests, including social isolation (runway test), novelty (open-field, novel object), or fear eliciting situations (forced human approach) [29,30]. At present, little is known about whether the individual test parameters measured in previous studies reflect the

behavioral differences manifested in the every-day lives of dairy cattle. Gibbons et al. [31] found a connection between the sociability of dairy cows measured in an individual runway test and behavioral measures of sociability in the home pen. Furthermore, MacKay et al. [28] found a relationship between neophobia and boldness in dairy cows measured in a novel arena and novel object test and their lying behavior in the home pen, which were derived from longer term tri-axial accelerometer data. These studies indicate a certain level of behavioral consistency between individual and group contexts.

Ohl and Putman [32] argued that in a social species, the welfare of an individual depends on the welfare of its group companions to some extent. In addition, it has been suggested that the personality of individuals may play a role in the formation and maintenance of animal social networks [33], and thus personality could also be a relevant factor when considering the social welfare of dairy cattle groups [34]. Therefore, given the possible link between individual personality and the welfare of the group, it is important to assess the manifestation of personality traits within a group context. Furthermore, recording robust behavioral parameters in a practical manner (i.e., a simple test in the home pen instead of laborious individual tests) might facilitate routine assessments of personality in future animal husbandry.

Therefore, in this study, we first aimed to investigate whether the behavioral parameters assessed in a traditional individual test using lactating Holstein cows could be captured in a more practical test performed under group housing conditions. To assess the contextual stability of behavior, we applied a novel object test, which was performed as an individual test and also in the home pen group. In addition, the temporal stability of the behavioral parameters was determined in repeated individual and group tests 6 months later. We also used selected individual test parameters to derive personality traits. We repeated this procedure after 6 months to evaluate whether the identified personality traits exhibited temporal stability, which is necessary for potential practical application.

## **Animals, materials, and methods**

## Animals and housing

Individual and within group behavioral tests were performed on adult lactating Holstein-Friesian cows in spring 2016 (March – April; parity range: 1 – 3, age range: 2.3 – 5.2 years, days in milk range: 11 – 509) and the tests were repeated in autumn 2016 (October – November; parity range: 1 – 4, age range: 2.3 – 5.2 years, days in milk range: 4 – 589). The cows were housed in three separate groups (in spring:  $G_1 = 11$  cows,  $G_2 = 14$  cows,  $G_3 = 15$  cows; in autumn:  $G_1 = 12$  cows,  $G_2 = 14$  cows,  $G_3 = 11$  cows) in a loose housing barn at the Leibniz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany). Each group area ( $21.5 \times 7.5$  m) contained 15 deep litter lying stalls with straw, two electronic water bins, and 10 electronic feed bins (Insentec RIC System, Hokofarm Group, Marknesse, Netherlands) where the total mixed ration was provided ad libitum. In all three groups every cow had access to all bins in the group and cows were slightly overstocked at feed bins. In the individual test situation, 39 cows were tested in the spring and 33 cows were retested in the autumn. In the group test situation 40 cows were tested in the spring, 38 of which also participated in the individual test, due to experimental reasons. In the autumn, 25 of the 40 cows were retested in the group test ( $G_1 = 7$  retested cows,  $G_2 = 8$  retested cows,  $G_3 = 10$  retested cows). The group composition changed slightly between spring and autumn mainly because cows left for the dry period and returned after calving. All of the cows were healthy and not in heat during the behavioral tests. All animal care and experimental procedures were performed in accordance with the German welfare requirements for farm animals and the ASAB/ABS Guidelines for the Use of Animals in Research [35]. All procedures involving animal handling and treatment (repeated individual and group behavioral tests) were approved by the Animal Welfare Committee of the Leibniz Institute for Farm Animal Biology (FBN) and by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Western Pomerania, Germany (Mecklenburg-Western Pomerania State Office for Agriculture, Food Safety, and Fishery; Reference number: 7221.3-2-033/15).

## Experimental procedure

## Individual test

We performed a combined arena test in a closed observational arena, which was previously unknown to the cows. In the arena (5 × 10 m) the cows did not have any visual or auditory contact with conspecifics, because it was located in a separate, sound-isolated building close to the barn. The arena contained a one-way mirror on one side to allow the supervision of the experiment from an adjacent room (cows could only see it from 5 m but they could not approach it) and the flooring of the arena was made of rubber mats without bedding. We assigned each cow to one of the nine test days based on a randomized design to facilitate statistical testing for known fixed effects (i.e., age and parity; see S1 Table). The arena test was performed on each test day between 7:00 am and 12:00 pm, as follows. A familiar person led a cow from the barn to the arena. The combined arena test comprised three consecutive parts: 1) a novel arena test (NA) where the cow spent 10 min alone in the arena; followed by 2) a novel object test (NO) (Part A in S1 Fig.) where an unknown object was lowered down from the ceiling and this was removed after 10 min; directly followed by 3) a novel human test (NH) where an unknown human in standardized clothing (white overalls, which were unknown to the cows and not used by the barn staff) entered and stood at the predefined position in the arena for 10 min. The arena was cleaned with a scraper between tests and with high pressure water at the end of the test day. Tests were recorded with two video cameras (Sony YC 3189, Sony Corp., Tokyo, Japan) installed at opposite ends of the arena and with a digital recorder (EDR HD-2H14/4H4, EverFocus Electronics Corp., New Taipei City, Taiwan). During the combined arena test, 73 behavioral parameters were recorded, as suggested in previous studies (see [29] for a review). The recorded behavioral parameters and their definitions are provided in Table 1. We did not record play behavior (commonly used parameter in calves) because it has not been observed during the test. Vocalization was recorded using the audio channel of the video recordings.

**Table 1.** Behavioral parameters recorded during the arena tests.

| Recording type     | Novel arena test   | Novel object test     | Novel human test     | Definition                                     |
|--------------------|--------------------|-----------------------|----------------------|--|
| <i>D, F, L, MD</i> |                    | <i>Object Look</i>    | <i>Human Look</i>    | Looking at the object/human                    |
| <i>D, F, L, MD</i> |                    | <i>Object Contact</i> | <i>Human Contact</i> | Actively touching the object/human             |
| <i>D, F, L, MD</i> | <i>Movement</i>    | Movement              | Movement             | Taking steps, walking or jumping               |
| <i>D, F, L, MD</i> | No movement        | No movement           | No movement          | Standing still, legs not moving                |
| <i>D, F, L, MD</i> | <i>Exploration</i> | Exploration           | Exploration          | Sniffing the wall or the floor of the arena    |
| <i>D, F, L, MD</i> | <i>Mirror</i>      | Mirror                | Mirror               | Looking in the direction of the one-way mirror |
| F                  | Urination          | Urination             | Urination            | Urinating                                      |
| F                  | Defecation         | Defecation            | Defecation           | Defecating                                     |
| F                  | Vocalization       | Vocalization          | Vocalization         | Vocalizing                                     |
| No. test parameter | 19                 | 27                    | 27                   |  |

The recorded parameters and recording types for each part of the arena test are shown. Recording types: duration (D) in s, frequency (F), latency (L) in s, and mean duration (MD) in s. The behavioral parameters and the corresponding types used for further analyses are shown in italics.

## Group test

We performed a novel object test with each group [36] in their home pen during the spring and autumn, as follows. A novel object was hanging in the middle of the walking alley (21.5 × 3.65 m) for 3 h (8:00 – 11:00 am) and the area around the object (Part B in S1 Fig) was recorded with a camera (Panasonic HDC-SD 600, Panasonic Corp., Osaka, Japan). The latency (s), duration (s) and frequency of active contacts with the novel object were determined as behavioral parameters for each cow.

## Settings and video analysis

In the individual and group tests, we changed the form but kept the color and size (~30 cm diameter) of the objects used constant in the test repetitions. We used a yellow round bowl and a rectangular tray in the individual test, and an orange-black ball and can

in the group test. The colors used in both test contexts were similar (see S1 Fig) and visible to the cows [37]. Video data were coded using Mangold Interact v15 (Mangold International GmbH, Arnstorf, Germany). All the video coding was conducted by one trained observer.

## Statistical Analysis

All of the analyses were performed in R version 3.4.2 [38] unless specified otherwise. The significance level was set to  $P < 0.05$ .

## Behavioral parameters

Our goal was to retain the recorded parameters that possibly reflected true individual behavioral differences in the reaction to novelty in our setup, and to reach the 5 animals to parameter ratio which is suggested as a minimum when applying principal component analysis (PCA) [39]. Hence we excluded behavioral parameters according to the following conditions: 1) high level of possible external influence (by discarding defecation, urination, and vocalization); 2) potential bias caused by the experimental setup (by discarding latencies due to possible discrepancies between test start and start of a behavior, and discarding environment related parameters in the NO and NH tests since in these tests the environment is not novel anymore); 3) high interdependency with other parameters (by discarding all recording types with the behavioral parameter “No movement”); and 4) small between-animal variability (by discarding the recording types with the mean duration and frequency for all parameters). Finally, to ensure that measurement types remained consistent between tests, we used seven behavioral parameters from the individual test (shown in italics in Table 1) and the object contact duration from the group test for further analysis.

In a preliminary analysis we tested whether the retained behavioral parameters are influenced by known effects. The effects of test day, age and parity were considered for the individual test parameters (duration of movement, exploration, mirror, object look, object contact, human look, and human contact) whereas the effects of group, age and parity were analyzed for the group test parameter (duration of object contact). The spring and autumn data sets were analyzed separately in view to the differences between

cows based on the following known fixed effects (S1 Table shows the raw data): 1) test day (1 – 9 in spring and autumn); 2) parity (1 – 3 in spring and 1 – 4 in autumn); and 3) age in days (in spring: parity 1: 830 – 859, parity 2: 1341 – 1903, parity 3: 1551 – 1841; in autumn: parity 1: 1034 – 1048, parity 2: 1703 – 2037, parity 3: 1586 – 2155, parity 4: 1793 – 2064). We observed that the ages of cows were very similar in the first parity, in contrast to the multiparous cows. Hence, only multiparous cows were considered to investigate the impact of age. In this analysis, we applied a linear model where the covariates were age, parity, and test day nested in parity. The fixed effects were tested with an F-Test, where age had no significant effect. Based on these results and due to the high similarity in age of the first parity cows, we excluded the covariate age from the analyses that considered all cows. Thus, the final linear model used to test all the individual test parameters included parity and test day nested in parity. The group test parameter object contact duration (see S2 Table for the raw data) was tested in a similar manner. Age had no impact for multiparous cows, so the final linear model only included the fixed effects of parity and group nested in parity. The final analysis was performed for all cows in spring and for the retested cows in autumn. The linear model analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) using the PROC MIXED function. We applied the post-hoc Tukey – Kramer test to correct for multiple testing.

**Stability between contexts:** We investigated whether the behavioral parameters measured in the time consuming individual test corresponded to the behavioral parameter object contact measured in the group test, which is a more practical parameter to measure. We hypothesized that there would be positive relationship between object contact duration in the group test and the individual test parameters: exploration, object contact, and human contact. First, the Spearman's rank correlation coefficients were calculated between the individual test parameters and the group test parameter in spring. We considered correlations:  $R_s \leq 0.40$  weak,  $0.40 < R_s \leq 0.80$  moderate, and  $R_s > 0.80$  strong [40]. In addition, for the behavioral parameters in the individual tests, cows below the 25% quartile were categorized as “low” and cows over the 75% quartile were categorized as “high” [41]. To test whether the cows in these categories differed in view to their object contact duration in the group context, we compared the group test results for the “high” and “low” categories using Wilcoxon’s rank-sum test.

**Stability over time:** The stability of the behavioral test parameters over time was determined for the individual test and for the group test using Spearman's rank correlation coefficients.

### **Personality traits**

The following analyses were performed using the R package psych [42]. We obtained personality traits via PCA with varimax rotation. PCA was performed using the seven individual test parameters obtained for the 39 cows tested in the spring. The suitability of our data set for PCA was confirmed with the measure of sampling adequacy using the Kayser – Meyer – Olkin criterion and Bartlett's sphericity test [39]. We used the Spearman's rank correlation matrix as input data (S3 Table) (following [23]), because the behavioral parameters were partly not normally distributed. Given the small sample size, we performed two additional PCAs to assess the component stability. In this analysis, we used the separate spring and autumn individual test results for the 33 cows that we tested twice. Tucker's congruence coefficient [43] was calculated using the loading matrices from all three PCAs to determine the similarity of the components from the different PCAs. The number of rotated components (RC) for extraction was determined by the Kaiser rule (components with an eigenvalue >1) and using Horn's parallel test [39]. We used the weights obtained together with the standardized behavioral parameters to calculate the RC scores for each cow. Furthermore, these weights were also used to predict the RC scores for the 33 cows that we retested in the autumn.

**Stability between contexts:** To test the manifestation of the measured personality traits in the group context, we calculated Spearman's rank correlation coefficients between the RC scores and group test results. In addition, cows, that exhibited a clear behavioral tendency for each personality trait were categorized as low (RC scores < -0.5) or high (RC scores > 0.5) according to a previously published definition [23]. We then compared the group test situation results for the cows in these categories using Wilcoxon's rank-sum test.

**Stability over time:** The temporal stability of an individual in terms of each personality trait was measured with the Spearman's rank correlation coefficient based on the corresponding spring and autumn RC scores. In addition, to test the stability of individuals

considering both personality traits, we calculated the distance (in standard deviations (SDs)) between the spring and autumn scores within the two-dimensional space. Using these distances, the cows were classified into three classes: distance < 1 SD, 1–2 SD, and > 2 SD (following [23]).

## Results

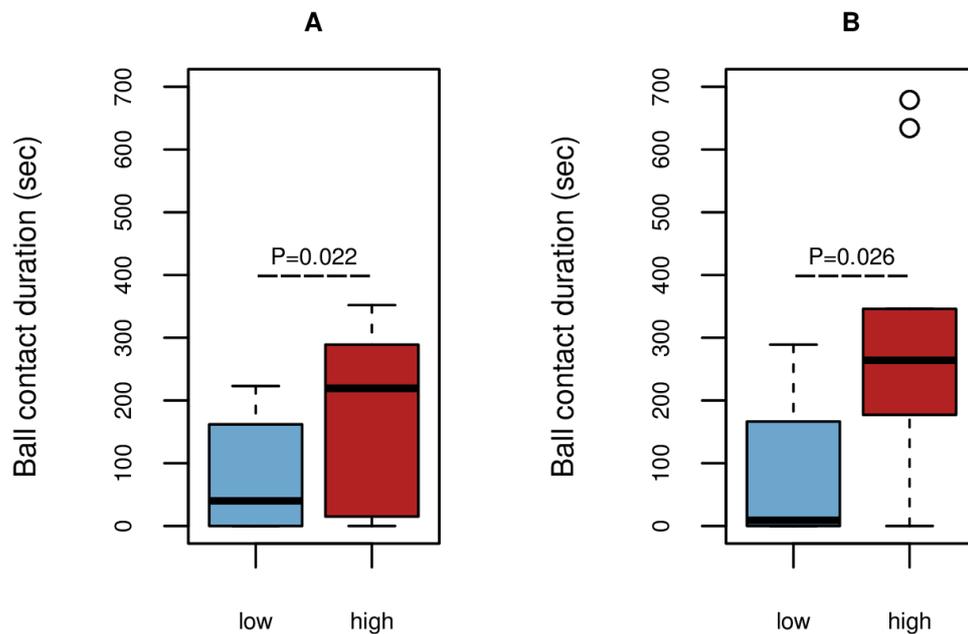
### Behavioral parameters

Descriptive statistics for the behavioral parameters are provided in S4 Table. The individual test parameter comprising object look was affected by parity in both seasons and by test day nested in parity in the spring (parity, spring:  $DF = 2$ ,  $F = 21.21$ ,  $P < 0.0001$ ; autumn:  $DF = 3$ ,  $F = 3.61$ ,  $P = 0.041$ ; test day nested in parity, spring:  $DF = 16$ ,  $F = 9.69$ ,  $P < 0.0001$ ; autumn:  $DF = 15$ ,  $F = 1.24$ ,  $P = 0.348$ ). This effect was due to the high object look value of one first parity cow in both seasons. We did not apply any correction because none of the other individual test parameters were affected and the sample size was small, with only four cows in their first parity (S1 Table). The analysis did not detect any significant effect of parity or group nested in parity for the group test parameter.

### Stability between contexts

There was a significant positive correlation between object contact in the group test and object contact ( $R_s = 0.361$ ,  $P = 0.026$ ) as well as movement ( $R_s = 0.336$ ,  $P = 0.039$ ) in the individual test. None of the other individual test parameters had significant correlations with object contact in the group test. Hence, we only used the individual test parameters comprising movement and object contact to classify cows as “low” or “high” and we tested for significant differences in the group test results. The corresponding boxplots are presented in Fig 1, which shows that both parameters had significant differences ( $P = 0.022$  for movement and  $P = 0.026$  for object contact). Cows classified as “high” by movement and “high” by object contact had longer object contacts in the group test than cows in the corresponding “low” categories.

**Fig 1.** Object contact durations of cows in the group test. Cows were categorized as low (< 25% quartile) and high (> 75% quartile) based on the parameters measured in the individual test: (A) movement duration, (B) object contact duration.



### Stability over time

In the individual test, the stability between test repetitions was moderate for movement ( $R_s = 0.422$ ,  $P = 0.015$ ), exploration ( $R_s = 0.401$ ,  $P = 0.021$ ), and human contact ( $R_s = 0.569$ ,  $P = 0.001$ ) and low for human look ( $R_s = 0.389$ ,  $P = 0.025$ ). The association between the spring and autumn results was negligible for the other behavioral parameters (range:  $R_s = 0.112 - 0.246$ ). For the group test, there was no correlation ( $R_s = -0.025$ ,  $P = 0.906$ ) between the spring and autumn results for the 25 cows that we tested twice.

### Personality traits

PCA was applicable because Bartlett's sphericity test rejected the hypothesis of all zero correlations ( $P < 0.001$ ) and the measure of sampling adequacy was  $> 0.5$  in all cases [39] (0.552 for spring, 39 cows; 0.575 for spring, 33 cows; 0.575 for autumn, 33 cows). In the spring PCA, three components had eigenvalues  $> 1$ , but only two in the autumn PCA. In addition, simulations using Horn's parallel test indicated the extraction of two components (S2 Fig). Based on these test results, two RCs were extracted.

The results of the three PCAs are presented in Table 2. In the first PCA (using all of the cows tested in the spring), the two extracted RCs explained 54.9% of the total variance. In the two other PCAs, using the spring and autumn values for the 33 cows that we tested twice, the RCs explained 53.1% and 55.4% of the total variance, respectively. The similarity of the corresponding RCs obtained from the three PCAs was assessed with Tucker's congruence coefficient, which indicated good similarity for all pairs (all values  $>0.94$ ; S5 Table). Furthermore, the Spearman's correlation coefficients between the RC scores from the two spring PCAs and between the predicted scores and autumn PCA scores were all higher than 0.95. We assigned personality trait names to the RCs based on the biological meanings of the behavioral parameters with "very good" loadings at least ( $>0.63$  or  $<-0.63$ ) [44]. RC1 was determined by the loadings for movement, exploration, and mirror, and it was termed activity/exploration. RC2 was determined by the loadings for object contact and human contact in spring and human look and human contact in autumn, and thus it was designated as boldness (Table 2).

**Table 2.** Loadings for the behavioral parameters and personality traits assigned to the obtained rotated components (RC).

| Parameter                | Spring (39 cows)         |                | Spring (33 cows)         |                | Autumn (33 cows)         |                |
|--------------------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|
|                          | RC1<br>(32.4%)           | RC2<br>(22.5%) | RC1<br>(32.1%)           | RC2<br>(21.0%) | RC1<br>(33.7%)           | RC2<br>(21.7%) |
| Movement                 | <i>0.919</i>             | 0.143          | <i>0.922</i>             | 0.138          | <i>0.876</i>             | 0.077          |
| Exploration              | <i>0.874</i>             | -0.103         | <i>0.838</i>             | -0.215         | <i>0.874</i>             | 0.050          |
| Mirror                   | <i>-0.755</i>            | -0.180         | <i>-0.761</i>            | -0.191         | <i>-0.768</i>            | -0.013         |
| Object Look              | -0.205                   | -0.486         | -0.235                   | -0.331         | -0.329                   | -0.345         |
| Object Contact           | -0.122                   | <i>0.669</i>   | -0.124                   | <i>0.688</i>   | 0.299                    | 0.562          |
| Human Look               | -0.006                   | -0.430         | 0.020                    | -0.478         | 0.198                    | <i>-0.715</i>  |
| Human Contact            | 0.187                    | <i>0.800</i>   | 0.210                    | <i>0.748</i>   | 0.044                    | <i>0.753</i>   |
| <b>Personality Trait</b> | Activity/<br>Exploration | Boldness       | Activity/<br>Exploration | Boldness       | Activity/<br>Exploration | Boldness       |

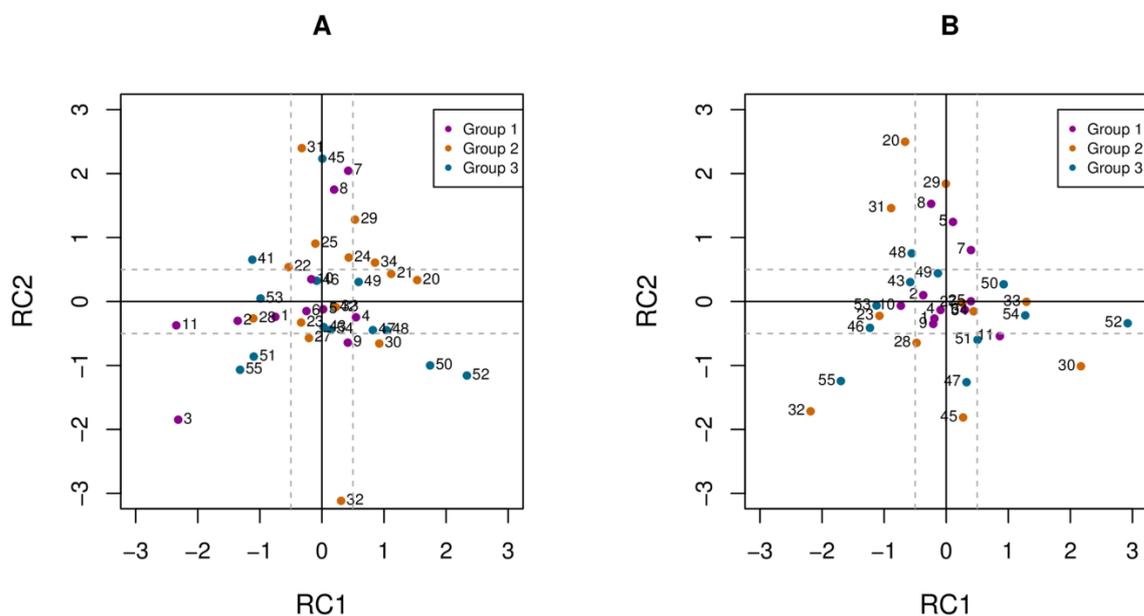
The percentage of variance explained for each RC is shown in parentheses. Parameters with high loadings ( $>0.63$  or  $<-0.63$ ) are shown in italics.

## Stability between contexts

In the spring, there was a weak positive correlation between object contact duration in the group test and the RC2 scores for the cows ( $R_s = 0.302$ ,  $P = 0.065$ ). In contrast to the classification using single test parameters, the classification based on RC scores as high and low did not indicate significant differences.

### Stability over time

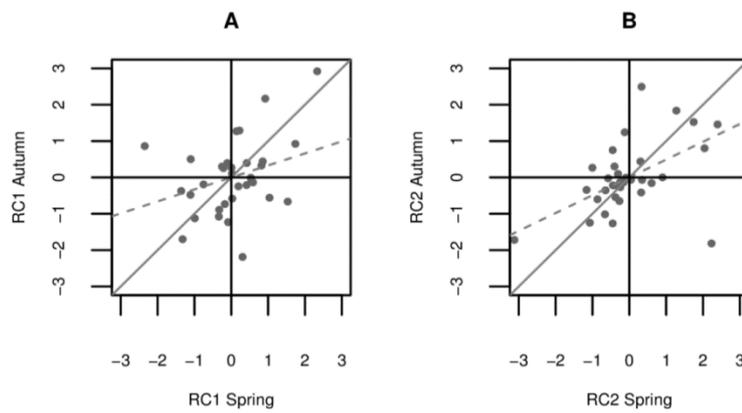
The positions of cows within the two-dimensional space based on the PCA conducted with 39 cows in the spring and the predicted RC scores in the autumn are presented in Fig 2.



**Fig 2.** Rotated component (RC1 and RC2) scores for cows in the spring (A; 39 cows) and predicted scores in the autumn (B; 33 cows). The analysis was performed in each season based on all cows, colors highlight the group assignment of cows.

Considering the stability within the two-dimensional space, in the repeated test 48.5% of the cows scored  $< 1$  SD, 39.4% between 1–2 SD, and 12.1%  $< 3$  SD distance from their spring scores (S3 Fig).

The stability of the RC scores between spring and autumn is shown in Fig 3. Not all of the individual test parameters were repeatable, but we found a positive association for both of the derived personality traits, where the correlations between the spring and autumn RC scores for cows were  $R_s = 0.334$  ( $P = 0.058$ ) for RC1 (activity/exploration) and  $R_s = 0.491$  ( $P = 0.004$ ) for RC2 (boldness).



**Fig 3.** Stability of the rotated component (RC) scores between spring and autumn for (A) RC1 and (B) RC2. Solid gray line represents 100% stability between tests. Dashed gray line is the trend line.

## Discussion

By definition, personality traits are individual behavioral characteristics that exhibit consistency over time and between contexts [13]. To obtain a better understanding of the contextual and temporal stability of behavior in adult lactating dairy cattle, we measured the consistency of behavioral parameters obtained in repeated individual and group test situations. In addition, individual arena test parameters were used to derive multiple personality traits via PCA. The stability of these personality traits over 6 months and their agreement with the group test results were also investigated.

## Behavioral parameters

Open field, novel object, and novel human tests have been used to assess behavioral variation in several species [18,29]. The sample size limits the number of variables that can be used for certain statistical analyses, such as PCA [39], so it is usual to discard some of the measured parameters from the analysis in animal personality research. To obtain parameters that provided the best possible descriptors of individual behavioral variation in the arena test, we recorded the commonly used parameters and subsequently applied a reduction procedure. Our goal was to use parameters that had the strongest relationships with the reactions of cows to a new situation. Furthermore, we selected parameters with possibly high variance in order to identify the characteristics of the behavioral reactions that differed between individuals (Table 1).

## Stability between contexts

In addition to the individual test, we performed a group novel object test within the home pen to measure whether this simple to use test could determine the same individual differences that are routinely measured in time-consuming individual tests. Behavioral tests that are performed in every-day environments might have more practical relevance but they can be influenced by factors that are difficult to control, e.g., the presence of group companions may lead to social facilitation [45,46] or they may hinder the access to the test object. Nevertheless, we found a positive association between object contact duration in the group test and movement duration in the NA test or object contact duration in the NO test (Fig 1). In a recent study, the novel object contact duration of calves was found to be moderately correlated with the feed variety preference in a forage test when the same animals were tested in the home pen as weaned heifers (however, heifers were tested one by one, while the other group members were held in another section of the pen) [47]. These results and those obtained in other studies [28,31] indicate that some aspects of the behavioral variations that can be observed in an individual test situation are also manifested in the group. In the future, repeated tests using a range of different stimuli (as suggested in [48]) might help to capture the consistent behavioral variations exhibited in the every-day lives of cattle.

## Stability over time

Four out of the seven individual test parameters showed agreement in the two tests conducted 6 months apart. These were both parameters related to the reaction to the new environment (movement duration and exploration duration) as well as parameters in the context with the appearance of an unknown person (human contact duration and human look duration). It is possible that the NA and NH parts of the arena test were the most stressful, and this might explain the stability of the reactions. In contrast to the NA and NH tests, the parameters measured in the NO test had negligible repeatability. Other studies obtained mixed results regarding the repeatability of the NO and NH test parameters (see [48] for a detailed discussion). It has been suggested that shorter intervals between tests and presenting the same object in the second test will generally improve the repeatability of the test results [48]. However, if our goal is to

obtain robust measures for describing the behavioral variation that is consistent over a longer time period, then it may be more beneficial to use personality traits derived from different behavioral parameters obtained in several tests.

The group test results were not consistent in the spring and autumn. In this context, it is important to note that the group composition changed between the two tests (S2 Table) due to calving. We could not assess the impact of cows that had their first test in the autumn, but it was possible to compare the group test results for 25 cows that we tested twice. Importantly, habituation could have caused the inconsistency of the results for the repeatedly tested cows because the object contact duration was considerably shorter in the autumn than in the spring (S4 Table). Similar habituation effects were found in a previous study with a repeated visual obstacle test using lactating cows, which was also conducted in a familiar environment [49]. In addition to habituation, the social environment might affect behavioral variations even in non-social behavior due to carry-over effects [50]. In our study, the number of cows in one group was smaller in the second test (S2 Table). Thus, in this group, the cows experienced less competition for other resources (feeder, lying stalls) in the autumn, which may have made more energy available for exploring the novel object. Our video observations in this group also suggest that the behavior of the dominant animals may have affected the group test results. Based on the individual values in this group (S2 Table), it is possible to speculate that two dominant animals may have blocked the object in the first test. Therefore in addition to the other reasons mentioned above, the presence or absence of specific cows in the autumn could explain the instability, thereby indicating the impact of the social structure on the expression of individual behavior.

## **Personality traits**

The arena test comprised a combination of commonly used individual behavioral tests (NA, NO, and NH) and it represented a stressful situation for the cows [27,51]. We expected that the behavioral reactions in the test situations would differ between individuals according to their personalities. We used different parameters from all three parts of the arena test in a PCA to determine the underlying structure of the behavioral variations. These behavioral parameters were selected using a systematic reduction

procedure to maintain the suggested five animals to parameter ratio, which is considered to be the minimum for using PCA [39]. PCA identified two main components, which were confirmed by two additional PCAs based on the spring and autumn results from the retested cows (Table 2). These results indicate that the two extracted components were stable although our data sets were small. These findings support the multidimensional nature of cattle personality described previously in calves [22-24] and they also indicate a stable personality trait structure in adult dairy cattle.

RC1 corresponded to the amount of time a cow spent with locomotion and exploration during the NA test. The mirror behavioral parameter, which was interpreted as inactive behavior because it comprised the time when a cow was standing still and looking in the direction of the one-way mirror in the arena, had a strong negative loading on RC1. Based on the high loading behaviors we associated RC1 with the activity/exploration personality trait. An analogous personality trait was also found in other studies of dairy calves [22,23,52-54] and cows [27]. In the framework proposed by Réale et al. [19], activity and exploration are considered to be different personality traits, but various other studies in cattle have shown that exploration and locomotion in a new environment loaded highly on the same component [23,27,55]. Overall, these findings indicate that these behaviors could have the same motivational background. In addition, locomotion by cows within a test arena was also suggested to represent fearfulness [27,28]. However, the reaction to an alarming situation can also be influenced by the coping style [56] of the animal, and fear may result in different (active or inactive) behavioral responses. Further investigations are required to determine whether these traits in dairy cattle are independent or linked, and if they form a behavioral syndrome. In our study, there was only a weak association between the RC1 scores obtained in the spring and autumn. Habituation to the test situation can lead to decreased locomotion and exploration in a novel environment [28,51], and cows do not exhibit dishabituation even after a long period [27]. In our study, the NA test situation remained completely unchanged between the test repetitions, so the low stability of the feature measured in this test phase might be explained by habituation.

RC2 was positively associated with the duration of contact with the object or human. Long contacts with the novel object or human correspond to risk-taking behavior,

and thus we associated RC2 with the boldness personality trait, which is described as the propensity to take risks [17,57]. The interpretation of this personality trait on the shyness–boldness continuum was further supported by the negative loadings of the parameters comprising object look duration and human look duration. Our results indicated that some shy cows looked for long periods at the unknown object or human, but they had little contact. The level of attention to the potential source of danger may also be determined by the anxiety of the cow as a distinct trait as well as by its boldness, which could explain the weak negative loadings for these parameters on RC2. We detected moderate stability of boldness after a 6 month period (Fig 3), thereby indicating the practical relevance of this trait in adult dairy cattle. These findings agree with previous studies that also identified a corresponding trait in cattle using open field and novel object tests in cows [28] and calves [22,23,54], although these studies employed shorter time periods between test repetitions.

A previous meta-analysis of studies that reported the repeatability of behavioral traits in non-domesticated animals determined an average repeatability of 0.37 [58]. Our results are in the same range for both of the personality traits identified in the present study. In calves, analogous personality traits showed slightly lower temporal stability, and the positions of the calves within the two-dimensional space (cf. S3 Fig) were also less consistent [23]. Behavior and reactivity might be more flexible during early ontogenesis [59,60], which could explain the higher stability that we found in adult dairy cattle compared with calves [61,62].

Despite the connection between the object contact durations in the individual and group tests, the association was weak between the boldness personality trait and novel object contact in the group test. The group test was conducted in the home environment with other group members present, so it was less stressful than the individual arena test, and thus it might have allowed for greater behavioral plasticity [26]. Furthermore, only one behavioral parameter was assessed in the group test, so it was probably less reliable than a personality trait derived from several measures. Automated data collection based on systems such as high-resolution location tracking could be employed in the future to obtain different behavioral parameters under group housing conditions over a longer time period. These measurements may be useful for studying the connections between social

behavior and personality, and they might broaden our knowledge on how behavioral variation is shaped by the environment.

## Conclusions

Overall, we found consistency between the single behavioral parameters measured in adult dairy cattle in two different contexts, i.e., individual and group tests. The repeated measurements after 6 months indicated the stability of most of the individual test parameters but not the group test parameter. Furthermore, based on the repeated measurements of individual behavior in a combined arena test, we identified two personality traits comprising activity/exploration and boldness, underlining the multidimensional nature of personality in cattle. These personality traits showed low to moderate stability after 6 months. The behavioral parameter measured in the group test only had a weak correlation with the corresponding personality trait. Overall, our results indicate that there is a relationship between the social environment and the manifestation of personality traits in every-day situations.

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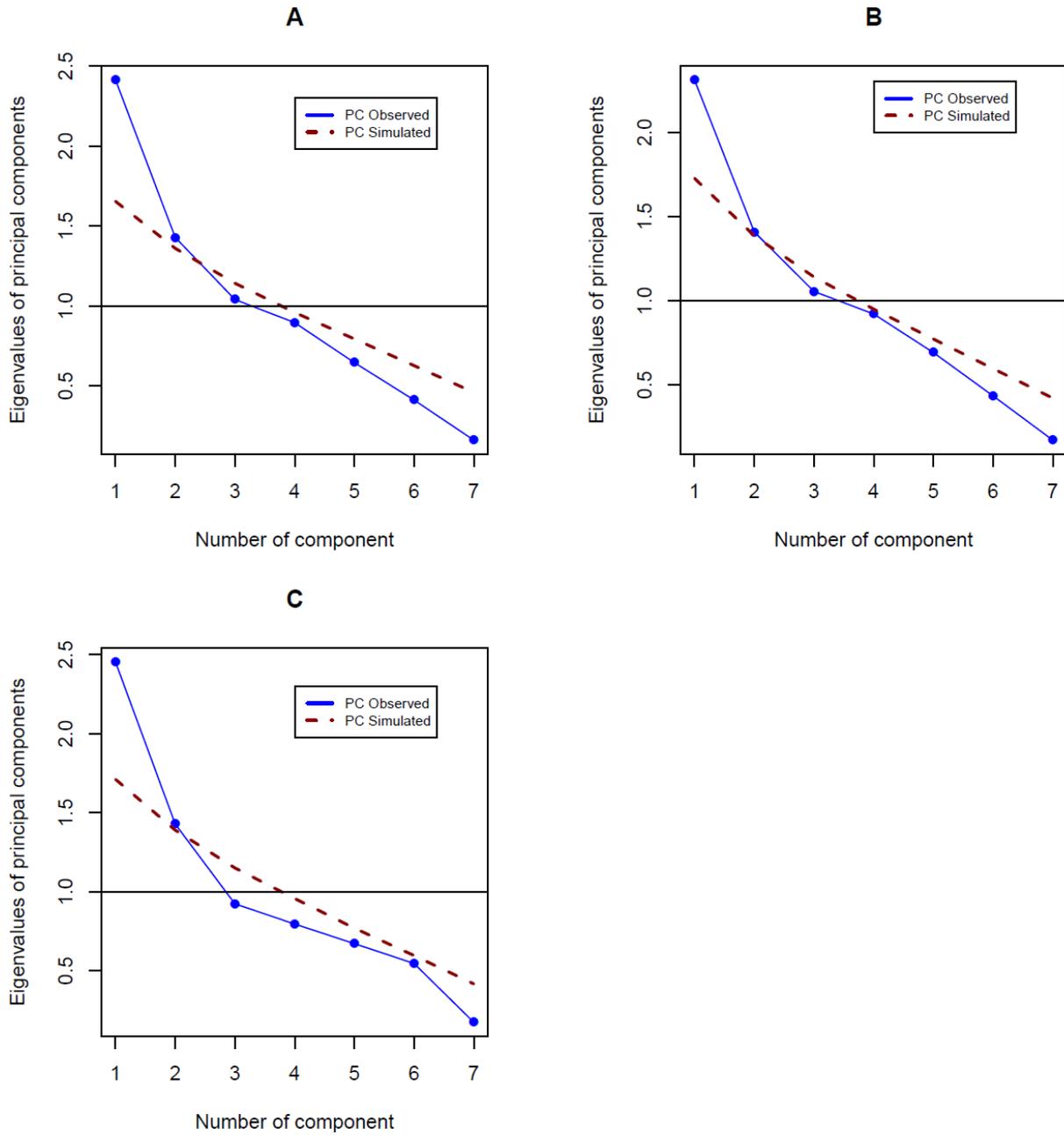
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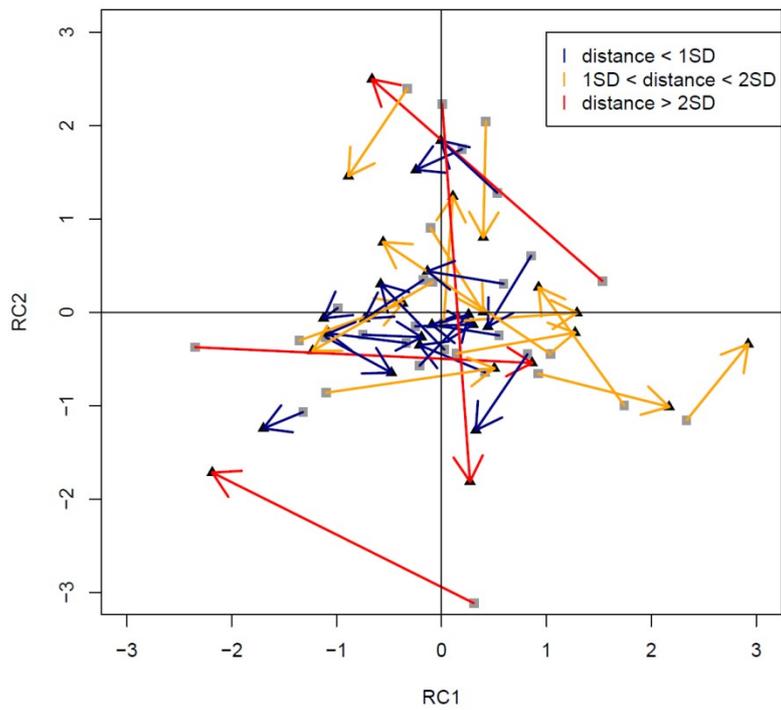
## Supporting information



**S1 Fig.** Novel object in the individual arena test (A) and group test (B).



**S2 Fig.** Results of Horn's parallel test: (A) spring, 39 cows; (B) spring, 33 cows and (C) autumn, 33 cows.



**S3 Fig.** Stability of the rotated component (RC1 and RC2) scores for 33 cows in the two-dimensional space. Positions of cows are represented by a gray square in the spring and by a black triangle in the autumn.

**S1 Table.** Arena test parameters used in the analysis and the corresponding fixed effects for each cow in the spring and autumn.

| Arena test, Spring |       |                         |                            |                       |                            |                               |                           |                              |          |            |        |
|--------------------|-------|-------------------------|----------------------------|-----------------------|----------------------------|-------------------------------|---------------------------|------------------------------|----------|------------|--------|
| Cow                | Group | Movement duration (sec) | Exploration duration (sec) | Mirror duration (sec) | Object Look duration (sec) | Object Contact duration (sec) | Human Look duration (sec) | Human Contact duration (sec) | Test day | Age (days) | Parity |
| 1                  | 1     | 142                     | 41                         | 96                    | 3                          | 1                             | 44                        | 0                            | 4        | 1597       | 2      |
| 2                  | 1     | 94                      | 46                         | 179                   | 30                         | 5                             | 33                        | 0                            | 8        | 1597       | 2      |
| 3                  | 1     | 85                      | 35                         | 413                   | 103                        | 10                            | 397                       | 0                            | 6        | 1519       | 2      |
| 4                  | 1     | 273                     | 134                        | 119                   | 30                         | 7                             | 68                        | 11                           | 6        | 1569       | 2      |
| 5                  | 1     | 192                     | 89                         | 46                    | 7                          | 7                             | 25                        | 0                            | 8        | 1593       | 2      |
| 6                  | 1     | 207                     | 108                        | 153                   | 41                         | 16                            | 53                        | 3                            | 8        | 1538       | 2      |
| 7                  | 1     | 265                     | 160                        | 70                    | 17                         | 63                            | 123                       | 30                           | 4        | 1585       | 2      |
| 8                  | 1     | 237                     | 124                        | 56                    | 8                          | 43                            | 50                        | 29                           | 4        | 1605       | 3      |
| 9                  | 1     | 238                     | 121                        | 100                   | 0                          | 6                             | 193                       | 4                            | 6        | 1562       | 3      |
| 10                 | 1     | 195                     | 72                         | 69                    | 27                         | 8                             | 93                        | 25                           | 8        | 1729       | 2      |
| 11                 | 1     | 97                      | 24                         | 407                   | 15                         | 9                             | 114                       | 0                            | 6        | 1561       | 3      |
| 20                 | 2     | 350                     | 188                        | 20                    | 8                          | 36                            | 97                        | 0                            | 1        | 830        | 1      |
| 21                 | 2     | 311                     | 156                        | 51                    | 14                         | 17                            | 76                        | 21                           | 1        | 1648       | 2      |
| 22                 | 2     | 126                     | 74                         | 58                    | 0                          | 15                            | 96                        | 18                           | 2        | 1824       | 2      |
| 23                 | 2     | 162                     | 138                        | 179                   | 13                         | 21                            | 159                       | 0                            | 1        | 1863       | 2      |
| 24                 | 2     | 254                     | 154                        | 150                   | 3                          | 16                            | 69                        | 29                           | 3        | 1639       | 2      |
| 25                 | 2     | 236                     | 79                         | 52                    | 23                         | 35                            | 19                        | 5                            | 1        | 1841       | 3      |
| 27                 | 2     | 189                     | 94                         | 129                   | 15                         | 3                             | 91                        | 0                            | 2        | 1826       | 2      |
| 28                 | 2     | 148                     | 41                         | 194                   | 18                         | 2                             | 45                        | 3                            | 2        | 833        | 1      |
| 29                 | 2     | 272                     | 107                        | 56                    | 7                          | 13                            | 41                        | 44                           | 2        | 1903       | 2      |
| 30                 | 2     | 273                     | 133                        | 33                    | 10                         | 5                             | 128                       | 0                            | 1        | 1606       | 2      |
| 31                 | 2     | 204                     | 94                         | 95                    | 9                          | 40                            | 68                        | 54                           | 3        | 1341       | 2      |
| 32                 | 2     | 210                     | 141                        | 83                    | 276                        | 0                             | 289                       | 0                            | 3        | 830        | 1      |
| 33                 | 2     | 231                     | 94                         | 73                    | 3                          | 7                             | 117                       | 11                           | 2        | 1684       | 2      |
| 34                 | 2     | 319                     | 131                        | 101                   | 21                         | 7                             | 48                        | 35                           | 3        | 1642       | 2      |
| 41                 | 3     | 158                     | 63                         | 243                   | 10                         | 9                             | 24                        | 25                           | 9        | 1571       | 2      |
| 42                 | 3     | 192                     | 125                        | 74                    | 1                          | 11                            | 89                        | 6                            | 7        | 1744       | 2      |
| 43                 | 3     | 190                     | 162                        | 197                   | 14                         | 7                             | 73                        | 7                            | 9        | 1681       | 2      |
| 45                 | 3     | 225                     | 116                        | 116                   | 14                         | 25                            | 41                        | 66                           | 5        | 1507       | 2      |
| 46                 | 3     | 236                     | 108                        | 147                   | 9                          | 19                            | 2                         | 3                            | 7        | 1630       | 2      |
| 47                 | 3     | 253                     | 169                        | 86                    | 18                         | 7                             | 25                        | 0                            | 9        | 1599       | 2      |
| 48                 | 3     | 268                     | 167                        | 55                    | 29                         | 3                             | 42                        | 8                            | 5        | 1680       | 2      |
| 49                 | 3     | 227                     | 160                        | 126                   | 0                          | 2                             | 158                       | 41                           | 5        | 1585       | 3      |
| 50                 | 3     | 339                     | 235                        | 120                   | 32                         | 0                             | 40                        | 1                            | 9        | 859        | 1      |
| 51                 | 3     | 126                     | 79                         | 209                   | 77                         | 14                            | 172                       | 0                            | 5        | 1767       | 2      |
| 52                 | 3     | 352                     | 255                        | 36                    | 4                          | 4                             | 174                       | 0                            | 9        | 1551       | 3      |
| 53                 | 3     | 130                     | 57                         | 162                   | 2                          | 8                             | 70                        | 7                            | 5        | 1543       | 2      |
| 54                 | 3     | 160                     | 134                        | 66                    | 19                         | 5                             | 72                        | 4                            | 7        | 1681       | 3      |
| 55                 | 3     | 85                      | 53                         | 185                   | 32                         | 16                            | 378                       | 0                            | 9        | 1747       | 2      |

| Arena test, Autumn |       |                         |                            |                       |                            |                               |                           |                              |          |            |        |
|--------------------|-------|-------------------------|----------------------------|-----------------------|----------------------------|-------------------------------|---------------------------|------------------------------|----------|------------|--------|
| Cow                | Group | Movement duration (sec) | Exploration duration (sec) | Mirror duration (sec) | Object Look duration (sec) | Object Contact duration (sec) | Human Look duration (sec) | Human Contact duration (sec) | Test day | Age (days) | Parity |
| 1                  | 1     | 138                     | 74                         | 114                   | 4                          | 0                             | 59                        | 0                            | 9        | 1835       | 3      |
| 2                  | 1     | 157                     | 57                         | 133                   | 1                          | 4                             | 30                        | 0                            | 4        | 1785       | 2      |
| 4                  | 1     | 180                     | 73                         | 129                   | 22                         | 1                             | 36                        | 9                            | 5        | 1765       | 3      |
| 5                  | 1     | 191                     | 69                         | 74                    | 0                          | 14                            | 53                        | 31                           | 5        | 1782       | 3      |
| 6                  | 1     | 259                     | 72                         | 116                   | 31                         | 9                             | 45                        | 0                            | 5        | 1727       | 2      |
| 7                  | 1     | 193                     | 121                        | 91                    | 0                          | 19                            | 85                        | 16                           | 5        | 1788       | 3      |
| 8                  | 1     | 156                     | 76                         | 107                   | 11                         | 15                            | 23                        | 43                           | 5        | 1808       | 3      |
| 9                  | 1     | 196                     | 78                         | 157                   | 42                         | 7                             | 39                        | 1                            | 9        | 1793       | 4      |
| 10                 | 1     | 151                     | 65                         | 220                   | 24                         | 0                             | 83                        | 23                           | 9        | 1953       | 3      |
| 11                 | 1     | 213                     | 114                        | 60                    | 0                          | 2                             | 178                       | 5                            | 4        | 1756       | 3      |
| 20                 | 2     | 190                     | 96                         | 162                   | 30                         | 56                            | 90                        | 32                           | 1        | 1048       | 1      |
| 23                 | 2     | 115                     | 49                         | 204                   | 27                         | 5                             | 38                        | 0                            | 3        | 2083       | 3      |
| 25                 | 1     | 195                     | 104                        | 81                    | 32                         | 6                             | 37                        | 13                           | 4        | 2064       | 4      |
| 27                 | 2     | 172                     | 64                         | 30                    | 9                          | 7                             | 83                        | 0                            | 1        | 2037       | 2      |
| 28                 | 2     | 169                     | 18                         | 80                    | 78                         | 3                             | 22                        | 2                            | 2        | 1045       | 1      |
| 29                 | 2     | 168                     | 70                         | 97                    | 23                         | 1                             | 40                        | 88                           | 9        | 2155       | 3      |
| 30                 | 2     | 314                     | 207                        | 40                    | 20                         | 8                             | 206                       | 0                            | 2        | 1825       | 3      |
| 31                 | 2     | 101                     | 41                         | 124                   | 27                         | 12                            | 51                        | 54                           | 9        | 1586       | 3      |
| 32                 | 2     | 24                      | 2                          | 242                   | 111                        | 0                             | 134                       | 0                            | 1        | 1034       | 1      |
| 33                 | 2     | 234                     | 143                        | 33                    | 2                          | 0                             | 42                        | 13                           | 1        | 1895       | 3      |
| 34                 | 2     | 237                     | 120                        | 161                   | 7                          | 4                             | 56                        | 5                            | 6        | 1853       | 3      |
| 43                 | 3     | 124                     | 97                         | 196                   | 16                         | 4                             | 24                        | 21                           | 7        | 1870       | 2      |
| 45                 | 2     | 210                     | 119                        | 167                   | 70                         | 0                             | 211                       | 0                            | 2        | 1703       | 2      |
| 46                 | 3     | 110                     | 58                         | 267                   | 8                          | 0                             | 78                        | 1                            | 8        | 1827       | 3      |
| 47                 | 3     | 135                     | 85                         | 33                    | 32                         | 0                             | 236                       | 0                            | 6        | 1787       | 3      |
| 48                 | 3     | 146                     | 76                         | 167                   | 2                          | 14                            | 56                        | 16                           | 9        | 1916       | 3      |
| 49                 | 3     | 180                     | 61                         | 119                   | 44                         | 3                             | 138                       | 53                           | 8        | 1789       | 3      |
| 50                 | 3     | 267                     | 121                        | 86                    | 8                          | 8                             | 27                        | 9                            | 6        | 1047       | 1      |
| 51                 | 3     | 173                     | 95                         | 43                    | 8                          | 9                             | 234                       | 0                            | 7        | 1970       | 3      |
| 52                 | 3     | 369                     | 256                        | 19                    | 10                         | 10                            | 129                       | 12                           | 6        | 1739       | 3      |
| 53                 | 3     | 67                      | 17                         | 129                   | 4                          | 2                             | 84                        | 2                            | 7        | 1746       | 3      |
| 54                 | 3     | 258                     | 158                        | 75                    | 32                         | 2                             | 66                        | 22                           | 7        | 1877       | 3      |
| 55                 | 3     | 40                      | 21                         | 223                   | 37                         | 6                             | 303                       | 0                            | 8        | 1937       | 3      |

**S2 Table.** Group novel object test results and fixed effects in spring (left) and autumn (right) for the three groups. Retested cows are marked in gray. Mean values for the retested cows are indicated by Mean rep.

**Group 1 Spring**

| Cow             | Object Contact (sec) | Age (days) | Parity |
|-----------------|----------------------|------------|--------|
| 5               | 274                  | 1605       | 2      |
| 6               | 476                  | 1550       | 2      |
| 7               | 177                  | 1611       | 2      |
| 8               | 679                  | 1631       | 3      |
| 9               | 589                  | 1581       | 3      |
| 10              | 0                    | 1741       | 2      |
| 11              | 173                  | 1580       | 3      |
| 1               | 0                    | 1623       | 2      |
| 2               | 0                    | 1609       | 2      |
| 3               | 223                  | 1538       | 2      |
| 4               | 15                   | 1588       | 2      |
| <b>Mean</b>     | 236,909              | 1605,182   | 2,273  |
| <b>SD</b>       | 234,218              | 50,947     | 0,445  |
| <b>Min</b>      | 0                    | 1538       | 2      |
| <b>Max</b>      | 679                  | 1741       | 3      |
| <b>Mean rep</b> | 338,286              |            |        |

**Group 1 Autumn**

| Cow             | Object Contact (sec) | Age (days) | Parity |
|-----------------|----------------------|------------|--------|
| 5               | 195                  | 1809       | 3      |
| 6               | 7                    | 1754       | 2      |
| 7               | 8                    | 1815       | 3      |
| 8               | 37                   | 1835       | 3      |
| 9               | 0                    | 1785       | 4      |
| 10              | 0                    | 1945       | 3      |
| 11              | 30                   | 1784       | 3      |
| 12              | 59                   | 1712       | 3      |
| 13              | 0                    | 1901       | 3      |
| 14              | 102                  | 2624       | 4      |
| 15              | 12                   | 1805       | 3      |
| 25              | 72                   | 2092       | 4      |
| <b>Mean</b>     | 43,5                 | 1905,083   | 3,167  |
| <b>SD</b>       | 55,462               | 237,145    | 0,553  |
| <b>Min</b>      | 0                    | 1712       | 2      |
| <b>Max</b>      | 195                  | 2624       | 4      |
| <b>Mean rep</b> | 39,571               |            |        |

**Group 2 Spring**

| Cow             | Object Contact (sec) | Age (days) | Parity |
|-----------------|----------------------|------------|--------|
| 26              | 0                    | 1681       | 3      |
| 27              | 13                   | 1849       | 2      |
| 28              | 162                  | 856        | 1      |
| 29              | 238                  | 1926       | 2      |
| 30              | 207                  | 1636       | 2      |
| 31              | 264                  | 1357       | 2      |
| 32              | 171                  | 846        | 1      |
| 33              | 357                  | 1707       | 2      |
| 20              | 346                  | 860        | 1      |
| 21              | 232                  | 1678       | 2      |
| 22              | 133                  | 1847       | 2      |
| 23              | 57                   | 1893       | 2      |
| 24              | 352                  | 1655       | 2      |
| 25              | 336                  | 1871       | 3      |
| <b>Mean</b>     | 204,857              | 1547,286   | 1,929  |
| <b>SD</b>       | 118,334              | 387,924    | 0,593  |
| <b>Min</b>      | 0                    | 846        | 1      |
| <b>Max</b>      | 357                  | 1926       | 3      |
| <b>Mean rep</b> | 176,5                |            |        |

**Group 2 Autumn**

| Cow             | Object Contact (sec) | Age (days) | Parity |
|-----------------|----------------------|------------|--------|
| 26              | 101                  | 1912       | 3      |
| 27              | 131                  | 2080       | 2      |
| 28              | 40                   | 1087       | 1      |
| 29              | 4                    | 2157       | 3      |
| 30              | 52                   | 1867       | 3      |
| 31              | 114                  | 1588       | 3      |
| 32              | 213                  | 1077       | 1      |
| 33              | 41                   | 1938       | 3      |
| 34              | 38                   | 1889       | 3      |
| 35              | 61                   | 1908       | 3      |
| 36              | 102                  | 1507       | 2      |
| 37              | 0                    | 1471       | 2      |
| 38              | 36                   | 2198       | 4      |
| 45              | 68                   | 1745       | 2      |
| <b>Mean</b>     | 71,5                 | 1744,571   | 2,5    |
| <b>SD</b>       | 54,541               | 343,309    | 0,824  |
| <b>Min</b>      | 0                    | 1077       | 1      |
| <b>Max</b>      | 213                  | 2198       | 4      |
| <b>Mean rep</b> | 87                   |            |        |

**Group 3 Spring**

| <b>Cow</b>      | <b>Object Contact (sec)</b> | <b>Age (days)</b> | <b>Parity</b> |
|-----------------|-----------------------------|-------------------|---------------|
| 46              | 0                           | 1616              | 2             |
| 47              | 0                           | 1578              | 2             |
| 48              | 0                           | 1673              | 2             |
| 49              | 0                           | 1578              | 3             |
| 50              | 289                         | 838               | 1             |
| 51              | 0                           | 1760              | 2             |
| 52              | 5                           | 1530              | 3             |
| 53              | 80                          | 1536              | 2             |
| 54              | 0                           | 1667              | 3             |
| 55              | 0                           | 1726              | 2             |
| 41              | 0                           | 1550              | 2             |
| 42              | 183                         | 1730              | 2             |
| 43              | 379                         | 1660              | 2             |
| 44              | 183                         | 1541              | 3             |
| 45              | 634                         | 1500              | 2             |
| <b>Mean</b>     | 116,867                     | 1565,533          | 2,2           |
| <b>SD</b>       | 182,145                     | 209,892           | 0,542         |
| <b>Min</b>      | 0                           | 838               | 1             |
| <b>Max</b>      | 634                         | 1760              | 3             |
| <b>Mean rep</b> | 37,4                        |                   |               |

**Group 3 Autumn**

| <b>Cow</b>      | <b>Object Contact (sec)</b> | <b>Age (days)</b> | <b>Parity</b> |
|-----------------|-----------------------------|-------------------|---------------|
| 46              | 151                         | 1844              | 3             |
| 47              | 69                          | 1806              | 3             |
| 48              | 36                          | 1901              | 3             |
| 49              | 9                           | 1806              | 3             |
| 50              | 83                          | 1066              | 1             |
| 51              | 27                          | 1988              | 3             |
| 52              | 313                         | 1758              | 3             |
| 53              | 152                         | 1764              | 3             |
| 54              | 6                           | 1895              | 3             |
| 55              | 0                           | 1954              | 3             |
| 56              | 163                         | 1827              | 3             |
| <b>Mean</b>     | 91,727                      | 1782,636          | 2,818         |
| <b>SD</b>       | 91,295                      | 237,319           | 0,575         |
| <b>Min</b>      | 0                           | 1066              | 1             |
| <b>Max</b>      | 313                         | 1988              | 3             |
| <b>Mean rep</b> | 84,6                        |                   |               |

**S3 Table.** Spearman's rank correlation matrices based on the individual test parameters used in three different principal component analyses. \*\*  $P < 0.05$ , \*  $P < 0.1$

| <b>Spring<br/>(39 cows)</b> | Movement  | Exploration | Mirror | Object<br>Look | Object<br>Contact | Human<br>Look | Human<br>Contact |
|-----------------------------|-----------|-------------|--------|----------------|-------------------|---------------|------------------|
| Movement                    | 1         |             |        |                |                   |               |                  |
| Exploration                 | 0.768 **  | 1           |        |                |                   |               |                  |
| Mirror                      | -0.618 ** | -0.427 **   | 1      |                |                   |               |                  |
| Object<br>Look              | -0,122    | -0,062      | 0.291* | 1              |                   |               |                  |
| Object<br>Contact           | 0,021     | -0,07       | -0,053 | -0,089         | 1                 |               |                  |
| Human<br>Look               | -0,142    | 0,071       | 0,066  | -0,016         | -0,031            | 1             |                  |
| Human<br>Contact            | 0.294 *   | 0,132       | -0,145 | -0.292 *       | 0.331 **          | -0,245        | 1                |

| <b>Spring<br/>(33 cows)</b> | Movement  | Exploration | Mirror | Object<br>Look | Object<br>Contact | Human<br>Look | Human<br>Contact |
|-----------------------------|-----------|-------------|--------|----------------|-------------------|---------------|------------------|
| Movement                    | 1         |             |        |                |                   |               |                  |
| Exploration                 | 0.721 **  | 1           |        |                |                   |               |                  |
| Mirror                      | -0.639 ** | -0.381 **   | 1      |                |                   |               |                  |
| Object<br>Look              | -0,139    | -0,009      | 0,262  | 1              |                   |               |                  |
| Object<br>Contact           | 0,005     | -0,119      | -0,036 | -0,052         | 1                 |               |                  |
| Human<br>Look               | -0,11     | 0,136       | 0,105  | -0,062         | -0,05             | 1             |                  |
| Human<br>Contact            | 0,282     | 0,086       | -0,132 | -0,185         | 0.297 *           | -0,192        | 1                |

| <b>Autumn<br/>(33 cows)</b> | Movement  | Exploration | Mirror | Object<br>Look | Object<br>Contact | Human<br>Look | Human<br>Contact |
|-----------------------------|-----------|-------------|--------|----------------|-------------------|---------------|------------------|
| Movement                    | 1         |             |        |                |                   |               |                  |
| Exploration                 | 0.780 **  | 1           |        |                |                   |               |                  |
| Mirror                      | -0.548 ** | -0.472 **   | 1      |                |                   |               |                  |
| Object<br>Look              | -0,115    | -0,236      | 0,252  | 1              |                   |               |                  |
| Object<br>Contact           | 0,245     | 0,18        | -0,187 | -0,206         | 1                 |               |                  |
| Human<br>Look               | -0,013    | 0,104       | -0,075 | 0,09           | -0,117            | 1             |                  |
| Human<br>Contact            | 0,086     | 0,145       | -0,038 | -0,096         | 0,26              | -0.297 *      | 1                |

**S4 Table.** Descriptive statistics for the behavioral parameters used in three different principal component analyses and in the group test. All of the behavioral parameters were measured in seconds.

| Individual Test         | Mean    | Median | SD      | Min | Max |
|-------------------------|---------|--------|---------|-----|-----|
| <b>Spring (39 cows)</b> |         |        |         |     |     |
| Movement Duration       | 211,564 | 210    | 72,528  | 85  | 352 |
| Exploration Duration    | 114,385 | 116    | 52,743  | 24  | 255 |
| Mirror Duration         | 123,179 | 100    | 87,458  | 20  | 413 |
| Object Look Duration    | 24,667  | 14     | 45,939  | 0   | 276 |
| Object Contact Duration | 13,385  | 8      | 13,558  | 0   | 63  |
| Human Look Duration     | 99,897  | 72     | 88,944  | 2   | 397 |
| Human Contact Duration  | 12,564  | 4      | 17,075  | 0   | 66  |
| <b>Spring (33 cows)</b> |         |        |         |     |     |
| Movement Duration       | 215,909 | 225    | 70,928  | 85  | 352 |
| Exploration Duration    | 116,788 | 116    | 53,482  | 24  | 255 |
| Mirror Duration         | 115,606 | 100    | 74,544  | 20  | 407 |
| Object Look Duration    | 25,182  | 15     | 47,461  | 0   | 276 |
| Object Contact Duration | 13,455  | 7      | 14,712  | 0   | 63  |
| Human Look Duration     | 95,303  | 70     | 79,889  | 2   | 378 |
| Human Contact Duration  | 11,848  | 3      | 17,972  | 0   | 66  |
| <b>Autumn (33 cows)</b> |         |        |         |     |     |
| Movement Duration       | 176,727 | 173    | 71,746  | 24  | 369 |
| Exploration Duration    | 87,182  | 76     | 52,018  | 2   | 256 |
| Mirror Duration         | 120,576 | 116    | 65,879  | 19  | 267 |
| Object Look Duration    | 23,394  | 20     | 24,707  | 0   | 111 |
| Object Contact Duration | 7       | 4      | 10,164  | 0   | 56  |
| Human Look Duration     | 91,394  | 59     | 73,929  | 22  | 303 |
| Human Contact Duration  | 14,273  | 5      | 20,349  | 0   | 88  |
| <b>Group Test</b>       |         |        |         |     |     |
| <b>Spring (40 cows)</b> |         |        |         |     |     |
| Object Contact Duration | 180,675 | 172    | 189,546 | 0   | 679 |
| <b>Autumn (25 cows)</b> |         |        |         |     |     |
| Object Contact Duration | 72,76   | 40     | 80,997  | 0   | 313 |

**S5 Table.** Tucker's congruence coefficients for the similarity of the rotated components (RC1 and RC2) obtained from three different principal component analyses.

|                  |     | Spring (39 cows) |             | Spring (33 cows) |             | Autumn (33 cows) |     |
|------------------|-----|------------------|-------------|------------------|-------------|------------------|-----|
|                  |     | RC1              | RC2         | RC1              | RC2         | RC1              | RC2 |
| Spring (39 cows) | RC1 | 1                |             |                  |             |                  |     |
|                  | RC2 | 0,18             | 1           |                  |             |                  |     |
| Spring (33 cows) | RC1 | <b>1</b>         | 0,2         | 1                |             |                  |     |
|                  | RC2 | 0,12             | <b>0,99</b> | 0,13             | 1           |                  |     |
| Autumn (33 cows) | RC1 | <b>0,94</b>      | 0,25        | <b>0,95</b>      | 0,18        | 1                |     |
|                  | RC2 | 0,15             | <b>0,95</b> | 0,15             | <b>0,94</b> | 0,15             | 1   |

## **2.2. Comprehensive analysis of affiliative and agonistic social networks in lactating dairy cattle groups**

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Borbala Foris designed and performed the experiment, analyzed the data, and wrote the manuscript with the support of and in agreement with her supervisor Dr. Nina Melzer and the co-authors of this manuscript.

## Comprehensive analysis of affiliative and agonistic social networks in lactating dairy cattle groups

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### Highlights:

1. Observation for 2–3 days is suitable for capturing social behaviour in dairy cows.
2. Displacement and grooming networks show different patterns.
3. A balance index was developed that combines affiliative and agonistic behaviours.
4. Affiliative and agonistic behaviour of cows was stable over 6 months.

### Abstract

The social environment of dairy cattle is important for their welfare under modern housing and management conditions. Social tension can negatively affect individuals even in a well-designed and healthy environment whereas affiliative behaviour may improve their well-being. The complex social relationships in a herd can be described comprehensively using network analysis. However, no up-to-date guidelines exist regarding the suitable time scale for assessing affiliative and agonistic behavioural structures in dairy cow groups. Dominance has been studied widely but the role of grooming and the relationships between affiliative and agonistic behaviours are still unclear. Furthermore, no measure exists that combines affiliative and agonistic interactions to describe the complete social experience of cows. In this study, we used video recordings and continuously assessed affiliative and agonistic interactions by all lactating Holstein cows in two groups (11 or 14 cows) during two periods (6 months apart). Based on the results of exploratory analysis for 5 days in one group, we aggregated the

interactions over 3 days in each group and period, and analysed directed and weighted social networks for the group as well as individual level assessments of affiliative and agonistic behaviours. The affiliative and agonistic networks had no correlations, indicating that the two behaviours followed different patterns. Individuals in the agonistic networks were tightly connected with several reciprocal displacement relationships, whereas they were loosely connected in affiliative networks and the majority of these relationships were asymmetrical. Cows showed high between-individual variability in terms of their number of interactions and partners, indicating that specific cows had different roles in both social networks. We developed a specific balance index to describe the complete social experience of cows based on received and given affiliative and agonistic interactions. A comparison of the balance index with grooming and dominance indices found no linear associations, which implies that combining affiliative and agonistic behaviours may provide different insights to grooming or dominance alone. All three indices showed a moderate to strong stability over 6 months in the subgroups of cows present during both observation periods, indicating that the cows had stable social behavioural characteristics. Our results highlight the importance of directed networks when studying social relationships in dairy cattle groups. We suggest that the combined analysis of affiliative and agonistic behaviours using a measure such as the balance index might help to better understand how the welfare of individuals is related to the group in which they live.

**Keywords:** affiliative, agonistic, dairy cow, social behaviour, social network, welfare

## 1. Introduction

The social environment is an important part of the every-day life of dairy cattle in loose housing barns and its relevance to animal welfare has been suggested in different contexts, where social stress due to frequent regrouping is a recognized concern (von Keyserlingk et al., 2008; Chebel et al., 2016) and the social environment might also be linked to illness (Proudfoot et al., 2012). Furthermore, social behaviour may be an indicator of the well-being of individual cows, where affiliative behaviour has been proposed as a positive welfare marker (Boissy et al., 2007; Napolitano et al., 2009; Rault,

2012) and changes in social behaviour might indicate impaired health (Galindo and Broom, 2000; Weary et al., 2009; Sepúlveda-Varas et al., 2016).

In recent years, technical development has yielded automated data collection methods for the practical assessment of social behaviour in cattle, such as proximity loggers (Boyland et al., 2013), electronic feeders (Huzzey et al., 2014), or location tracking systems (Gygax et al., 2007). However, appropriate validation is needed (Croft et al., 2016) before the routine application of automatically collected data instead of observing social interactions (Farine, 2015). In addition, it is necessary to determine a suitable time scale (Pinter-Wollman et al., 2014) that provides meaningful snapshots of social behaviour. Currently no up-to-date guidelines exist for dairy cattle regarding the time scale required to determine the social structure of groups because most studies conducted limited sampling in time and space, or considered focal animals. Therefore, continuous video observation of affiliative and agonistic interactions in groups over multiple days could provide sampling guidelines to facilitate future analyses as well as having implications for the validation of automated data collection methods.

Agonistic social interactions have been widely studied in dairy cattle (Wierenga, 1990; Val-Laillet et al., 2008) but most previous investigations considered dyadic relationships and the dominance hierarchy (Langbein and Puppe, 2004). Compared with dominance, less is known about the roles of affiliative behaviour in cattle. Affiliative grooming is known to be associated with: 1) positive emotions and improved coat hygiene (Boissy et al., 2007), 2) the formation of social bonds (Wasilewski, 2003; Gutmann et al., 2015), and 3) a calming effect in receiver cows (Laister et al., 2011). However, the association between the dominance rank and grooming behaviour of individual cows is not clear (Sato et al., 1993; Val-Laillet et al., 2009; Šárová et al., 2016), and the reported results are inconsistent regarding the connection between affiliative and agonistic behavioural patterns in cattle groups (Val-Laillet et al., 2009; Tresoldi et al., 2015). In a welfare context, the complete social experience of a cow within a group might be more important than solely agonistic or affiliative interactions. Therefore, the joint analysis of affiliative and agonistic behaviours using a common framework may facilitate the development of a comprehensive measure of the social experience of individual cows.

Social network analysis (SNA) is a suitable framework for investigating complex social relationships in a group and it has recently attracted increased attention from animal science researchers (Wey et al., 2008; Makagon et al., 2012), although relatively few studies have applied SNA to dairy cattle groups (e.g., cows by Gygas et al. (2010), Boyland et al. (2016); and calves by Koene and Ipema (2014), Chen et al. (2015)). All of these studies were based on automated data collection methods, and thus they inferred the relationships between cows and did not provide information about the actual social interactions.

In this study, we aimed to conduct detailed analysis of social behaviour in lactating dairy cattle groups based on continuous video observations of affiliative and agonistic interactions over multiple days. First, we investigated the most suitable time scale for detailed analysis of social behaviour. Second, we applied SNA to describe the social structure of the groups and to explore the relationship between affiliative and agonistic behaviour. In addition, we developed a new index to characterize the social experience of individual cows based on received and given interactions, and compared this index with dominance and grooming measures. Finally, we also assessed the consistency of social behavioural patterns over 6 months in the stable subgroups of cows present during both observation periods.

## **2. Animals, material, and methods**

### **2.1 Animals, housing, and data collection**

We observed two lactating Holstein cattle groups at the experimental cattle facility of the Leibniz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany). **Table 1** provides an overview of the cows and observation periods. The cows were housed in a free stall barn with a slatted floor and deep litter lying stalls with straw bedding. Each group area (21.5 × 7.5 m, **S1 Fig.**) contained 15 lying stalls, an automatic brush, two electronic water bins, and 10 electronic feeder bins (Insentec RIC System, Hokofarm Group, Marknesse, Netherlands), where the total mixed ration was provided ad libitum. Cows were milked twice daily (around 05:00 h and 16:00 h) and each group went to the milking parlour for approximately 30 min. Observations were performed in spring (February–March) and autumn (October–November) during 2016. We recorded each group using two video

cameras (Sony YC 3189, Sony Corp., Tokyo, Japan) and a digital recorder (EDR HD-2H14/4H4, EverFocus Electronics Corp., New Taipei City, Taiwan). Observations were performed in both groups on the same weekdays when all cows were healthy and none of them were in heat. To facilitate identification, cows were marked with an individual alphanumeric on their shoulder and flank using hair dye. There were no mother–daughter relationships between the observed cows. We assumed that the cows within the groups knew each other because after calving, the cows went back to the same group they occupied before the dry period, and no first parity cows were introduced during our study.

**Table 1.** Number of cows and days analysed within each group and season. For age, parity, and days in milk (DIM) by cows, the mean and the range (in parentheses) are provided.

| Group         | No. of cows          | No. of days | Age (years)     | Parity    | DIM            |
|---------------|----------------------|-------------|-----------------|-----------|----------------|
| <b>Spring</b> |                      |             |                 |           |                |
| Group 1       | 14                   | 3           | 4.3 (2.3 – 4.8) | 2 (1 – 3) | 293 (29 – 525) |
| Group 2       | 14                   | 3           | 4.2 (2.3 – 5.2) | 2 (1 – 3) | 233 (21 – 391) |
| <b>Autumn</b> |                      |             |                 |           |                |
| Group 1       | 14 (8) <sup>1</sup>  | 3           | 4.8 (2.9 – 6.0) | 2 (1 – 4) | 241 (4 – 573)  |
| Group 2       | 11 (10) <sup>1</sup> | 5           | 4.9 (3.0 – 5.5) | 3 (1 – 3) | 191 (44 – 357) |

<sup>1</sup>Number of repeatedly observed cows in the group.

The study was carried out according to the animal care guidelines of the state Mecklenburg-Western Pomerania, Germany, and it was approved by the Mecklenburg-Western Pomerania State Office for Agriculture, Food Safety and Fishery (Reference number: 7221.3-2-033/15).

## 2.2 Video observations

Affiliative and agonistic interactions were recorded in the whole group area for every cow based on continuous video observations (24 h each day) according to the following definitions (Haas, 2018): *actor*: cow initiating a social interaction; *receiver*: cow receiving a social interaction; *displacement*: agonistic behaviour comprising an aggressive contact that resulted in the receiver leaving its location (leaving the feeder/lying stall or taking a minimum of two steps in the walking alley); *grooming*: affiliative behaviour comprising an actor licking the receiver for a minimum of 15 s when both cows were in the same zone;

and *zone*: defined for each interaction based on the location of the head of the receiver cow, where the zones comprised: feeder and water bins together as the feeder, walking alley including the brush area, and lying stalls (**S1 Fig.**). Two interactions between the same actor and receiver were treated as one interaction if there was a break of less than 20 s between them and they occurred in the same zone. Only times when the group was undisturbed and all cows were present were considered in the analysis. In total, approximately 60–90 min were excluded each day, when the cows went for milking and due to occasional short term interruptions (cleaning and normal management activities). Video recordings were analysed by two trained observers using Mangold Interact V15 (Mangold International GmbH, Arnstorf, Germany) where each observer analysed the video recordings of one group.

## 2.3 Statistical analysis

All statistical analyses were performed in R version 3.4.2 (R Core Team, 2017). The significance level was set to  $P = 0.05$ .  $P$ -values were corrected for multiple testing via the Benjamini and Hochberg (Hochberg and Benjamini, 1990) correction method using the R function “p.adjust”.

### 2.3.1 Spatiotemporal distribution and stability of social interactions

We performed exploratory analysis of the social interactions over five successive days in one group during the autumn (**Table 1**). First, we assessed the frequency of displacement and grooming in each zone during each day. We also determined the number of social interactions that occurred during the day-time (05:30 h to 17:30 h) and night-time (17:30 h to 05:30 h) corresponding to the management activities in the barn. Second, in order to determine an appropriate time scale for the meaningful analysis of social behavioural patterns in the group, we assessed the stability of grooming and displacement by calculating the correlations for actor–receiver matrices via the permutation-based quadratic assignment procedure (QAP test) as implemented in the R package “sna” (Butts, 2016) with 10,000 resamples, according to Koene and Ipema (2014) and Chen et al. (2015). The QAP correlation ( $R_{QAP}$ ) was calculated between daily interaction matrices as well as between aggregated matrices containing interactions from two consecutive days, where only non-overlapping periods were considered (e.g., days 1 + 2 compared to days

3 + 4). Finally, we investigated how the number of actor–receiver pairs without interactions (zero values in the matrix) declined as the number of days considered increased.

### **2.3.2 Analysis of affiliative and agonistic networks**

Based on the exploratory analysis, in each group and season, three successive days were used for the detailed analysis of affiliative and agonistic behaviour. We created aggregated actor–receiver matrices using the grooming and displacement frequencies obtained from the 3 days. SNA (see Farine and Whitehead (2015) for a detailed description of the method) was used to describe the individual and group level patterns of grooming and displacement, and to explore the connection between the two behaviours. Briefly, in a social network, the nodes represent the individuals and the directed edges represent the interactions between them. Edges can be binary when only the existence of a connection is considered, or weighted when the strength of the connection is also indicated. In the case of directed networks, the edge goes from the actor to the receiver. Based on the aggregated actor–receiver matrices, we created directed and weighted social networks separately for grooming and for displacement.

We calculated the correlations between the aggregated grooming and displacement matrices using the QAP test. In addition, for each network, we calculated the standard network and node level measures (**Table 2**) using the R package “igraph” (Csardi and Nepusz, 2006).

**Table 2.** Definitions of the calculated network level and node level measures.

|               | Measure       | Definition   |
|---------------|---------------|--|
| Network level | Density       | Percentage of existing connections within the network  |
|               | Dyadic census | Number of mutual, asymmetric and null relationships  |
| Node level    | IN-Degree     | Number of incoming edges for a node;<br>number of partners from which a cow received an interaction  |
|               | OUT-Degree    | Number of outgoing edges for a node;<br>number of partners with which a cow initiated an interaction |
|               | IN-Strength   | Sum of incoming edge weights for a node;<br>number of interactions that a cow received               |
|               | OUT-Strength  | Sum of outgoing edge weights for a node;<br>number of interactions that a cow initiated              |

### 2.3.3 Indices of social behaviour

In order to make a straightforward comparison of the affiliative and agonistic behaviours of individual cows, we calculated three different social behaviour indices and compared them. The indices were calculated using the aggregated actor–receiver matrices containing data from 3 days.

#### 2.3.3.1 Dominance score (DS)

We determined DS values for cows using the normalized David's score (de Vries et al., 2006), which was calculated with the dyadic dominance index corrected for chance using the R package “steepness” (Leiva and de Vries, 2014). We used David's score because it is suitable for hierarchies that are not extremely steep according to a recent simulation study (Sánchez-Tójar et al., 2017). In addition, we followed the suggestions given in the same study regarding the benchmark of the interactions to animals ratio (10–20) for reliable dominance hierarchies.

#### 2.3.3.2 Grooming index (GI)

DS relies only on agonistic interactions so we aimed to obtain a similar index based solely on affiliative interactions. Therefore, using the grooming matrix, we calculated a GI as the ratio of received grooming to all grooming related to a cow, in a similar manner to Parr et al. (1997). GI ranges between 0 and 1, where values above 0.5 indicate that more grooming was received than performed and values below 0.5 indicate the opposite.

### 2.3.3.3 Balance index (BI)

We propose a new index called BI to represent the complete social experience of cows by considering both agonistic and affiliative interactions. BI expresses the relationship between the social behaviour that a specific cow receives (IN) and the social behaviour that this cow initiates (OUT) within the group. BI is calculated based on node level social network measures as follows:

$$BI = \frac{\frac{IN - Grooming + c}{IN - Displacement}}{\frac{OUT - Grooming + c}{OUT - Displacement}},$$

where IN-Grooming/IN-Displacement is either the number of grooming/displacement events a specific cow receives (calculated with IN-Strength) or the number of group members that groom/displace the specific cow (calculated with IN-Degree), and OUT-Grooming/OUT-Displacement is determined in the same manner. If we only consider the aforementioned measures in the ratio, several different combinations all result in BI = 1 but they differ regarding the IN and OUT measures. To differentiate between these cases, a small weight ( $c = 0.005$ ) is applied to the grooming measures. In cases where a cow is not the initiator or recipient of any affiliative or agonistic interactions, 0 values are replaced with 0.5 when calculating the ratio for the specific cow. In order to facilitate the interpretation of BI, after calculating the ratio, values smaller than one are transformed to their negative reciprocal. Furthermore, 1 is transformed to 0. BI can then be interpreted as follows. If BI = 0, the incoming and outgoing social behaviour is balanced, and the cow reacts to the group in the same manner as the group reacts to it. If  $BI \leq -1$ , the cow is in negative imbalance, and thus the incoming affiliative to agonistic behaviour ratio is lower than the outgoing ratio. Finally, if  $BI \geq 1$ , the cow is in positive imbalance, so the incoming affiliative to agonistic behaviour ratio is higher than the outgoing ratio. In the appendix, **S1 Document** provides further details regarding the calculation and the range of BI, as well as an example of the effect of the correction weight and replacing 0 values. In our analysis, we considered the signs of BI values and the BI ranks of cows within a group.

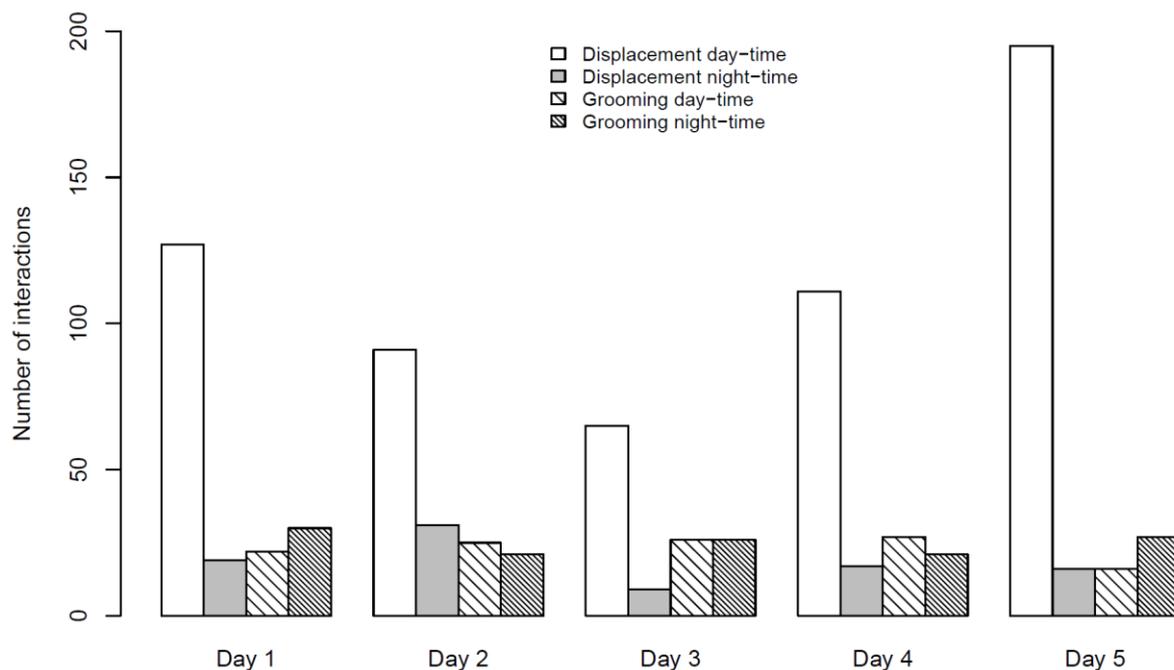
### 2.3.4 Long-term stability of social behaviour

Finally, we analysed the long-term stability of social behaviour using 3 days of observation during each season within the stable subgroups of cows present in both seasons in Group 1 (Subgroup 1: eight cows) and Group 2 (Subgroup 2: 10 cows). First, we calculated the correlations between the spring and autumn interaction matrices for affiliative and agonistic behaviour using the QAP test. Second, we calculated DS, GI, and BI within the subgroups and compared the spring and autumn values based on the Spearman's rank correlation coefficient ( $R_s$ ).

### 3. Results

#### 3.1 Spatiotemporal distribution and stability of social interactions

Based on continuous video analysis of five consecutive days in one group, we determined considerable differences between the daily displacement frequencies whereas the frequencies of grooming were relatively stable (**S1 Table**). On average, we observed  $48.2 \pm 3.9$  grooming and  $136.2 \pm 49.6$  displacement events per day. **Fig. 1** shows the frequency of grooming and displacement occurrences each day during the day-time and night-time. The days differed in terms of the total number of displacements but most displacements were observed during the day-time on each day. Furthermore, the feeder zone had the highest displacement frequency each day (**S1 Table**). By contrast, grooming was evenly distributed throughout the day where most grooming occurred at the lying stalls with a high frequency in the walking alley (**S1 Table**).



**Fig. 1.** Number of displacements and grooming events over five consecutive days during the autumn in Group 2 (11 cows). Each day was divided into day-time from 05:30 h to 17:30 h and night-time from 17:30 h to 05:30 h.

Regarding the stability of the daily grooming and displacement matrices, we observed a positive correlation between all days for both behaviours, but not all of these relationships were significant. The daily grooming matrices had a mean  $R_{QAP}$  value of 0.441 ( $R_{QAP}$  range: 0.293–0.682), whereas the displacement matrices had a mean  $R_{QAP}$  value of 0.409 ( $R_{QAP}$  range: 0.153–0.525). Correlations were higher when we considered the aggregated interaction matrices with 2 days of data, where there was a significant positive correlation between the interaction matrices for grooming ( $R_{QAP}$  range: 0.540–0.629, all corrected  $P < 0.01$ ) as well as for displacement ( $R_{QAP}$  range: 0.468–0.610, all corrected  $P < 0.01$ ). The declines in the number of actor–receiver pairs without interactions as the number of days considered increased are presented in **S2 Fig.** for grooming and displacement. We observed that the number of unknown relationships declined sharply only during the first few days, where a slightly slower decrease was observed for grooming. This analysis demonstrates that 2 days of continuous observations can provide sufficient information regarding the social structure in the groups. Therefore, in order to ensure the reliability of our data sets, we included an additional day and used 3 days of observations for each group and season in our subsequent analyses.

### 3.2 Analysis of affiliative and agonistic networks

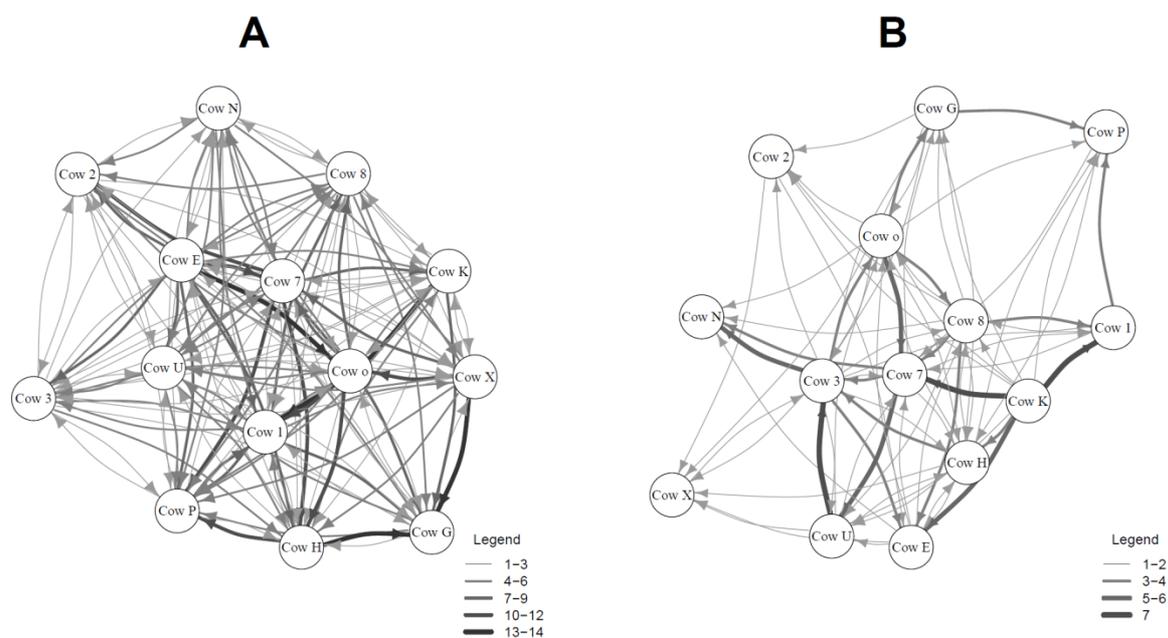
Based on the results of the exploratory analysis, the detailed analysis of social behaviour was performed using an aggregated data set comprising 3 days from each group and season. The numbers and percentages of observed affiliative and agonistic interactions as well as their locations are presented in **Table 3**. In general, we observed approximately two to six times more displacements than grooming events in the groups during both seasons. In Group 1 during the autumn, the number of displacements declined by 6.1% and the number of grooming events increased by 22.9% compared with the spring. In Group 2 during the autumn, three fewer cows were present and displacements declined by 42% compared with the spring, and 40.2% more grooming events were observed. Similar to the results of the exploratory analysis, in both seasons and groups, the majority of the displacements occurred at the feeder, followed by the walking alley and lying stalls. Grooming was evenly distributed between the lying stalls and the walking alley in Group 1, whereas the majority of grooming occurred in the lying stalls in Group 2.

**Table 3.** Number, percentage, and location of social interactions over three consecutive days in each group and season.

| Season | Group                | Displacement                 |                |                |                | Grooming                     |               |                |
|--------|----------------------|------------------------------|----------------|----------------|----------------|------------------------------|---------------|----------------|
|        |                      | Total                        | Feeder         | Lying stalls   | Walking alley  | Total                        | Lying stalls  | Walking alley  |
| Spring | Group 1<br>(14 cows) | <b>610</b><br><b>(78.6%)</b> | 374<br>(61.3%) | 124<br>(20.3%) | 112<br>(18.4%) | <b>166</b><br><b>(21.4%)</b> | 80<br>(48.2%) | 86<br>(51.8%)  |
|        | Group 2<br>(14 cows) | <b>590</b><br><b>(84.6%)</b> | 467<br>(79.2%) | 40<br>(6.8%)   | 83<br>(14.0%)  | <b>107</b><br><b>(15.4%)</b> | 67<br>(62.6%) | 40<br>(37.4%)  |
| Autumn | Group 1<br>(14 cows) | <b>573</b><br><b>(73.7%)</b> | 346<br>(60.4%) | 68<br>(11.9%)  | 159<br>(27.7%) | <b>204</b><br><b>(26.3%)</b> | 98<br>(48.0%) | 106<br>(52.0%) |
|        | Group 2<br>(11 cows) | <b>342</b><br><b>(69.5%)</b> | 278<br>(81.3%) | 22<br>(6.4%)   | 42<br>(12.3%)  | <b>150</b><br><b>(30.5%)</b> | 96<br>(64.0%) | 54<br>(36.0%)  |

Based on the affiliative and agonistic interactions, we created and analysed the directed and weighted social networks. **Fig. 2** shows an example of the agonistic and affiliative networks in Group 1 during the spring. The network level measures for social behaviour are presented in **Table 4**. The QAP test did not detect any correlations between the

affiliative and agonistic networks in the groups ( $R_{QAP}$  range:  $-0.060$  to  $0.083$ ). In Group 1, the agonistic network had a high density in both seasons and both cows displaced each other in most dyads. By contrast, in Group 2, the agonistic network density was lower in both seasons and the majority of the dyadic displacement relationships were asymmetric. In both groups and seasons, the affiliative networks had a lower density than the agonistic networks, where a considerable amount of dyads lacked any grooming events and the majority of the grooming relationships were asymmetric. The density of the affiliative networks was higher during the autumn than the spring in both groups, with a greater increase in Group 2. In Group 2, the percentage of mutual grooming relationships was also three times higher during the autumn than the spring.



**Fig. 2.** Examples of an agonistic (A) and affiliative (B) social network in Group 1 during the spring. The networks are based on interaction frequencies over 3 days. The arrows point from the actor to the receiver, whereby the line thickness represents the number of interactions.

**Table 4.** Network density, number, and percentage of mutual, asymmetric, and null relationships in the network in each group and season.

| Displacement    | No. of cows | Density | Mutual     | Asymmetric | Null       |
|-----------------|-------------|---------|------------|------------|------------|
| Group 1 Spring  | 14          | 0.813   | 58 (63.7%) | 32 (35.2%) | 1 (1.1%)   |
| Group 2 Spring  | 14          | 0.588   | 23 (25.3%) | 61 (67.0%) | 7 (7.7%)   |
| Group 1 Autumn  | 14          | 0.736   | 48 (52.7%) | 38 (41.8%) | 5 (5.5%)   |
| Group 2 Autumn  | 11          | 0.618   | 15 (27.3%) | 38 (69.1%) | 2 (3.6%)   |
| <b>Grooming</b> |             |         |            |            |            |
| Group 1 Spring  | 14          | 0.429   | 18 (19.8%) | 42 (46.2%) | 31 (34.1%) |
| Group 2 Spring  | 14          | 0.280   | 9 (9.9%)   | 33 (36.3%) | 49 (53.8%) |
| Group 1 Autumn  | 14          | 0.516   | 21 (23.1%) | 52 (57.1%) | 18 (19.8%) |
| Group 2 Autumn  | 11          | 0.509   | 18 (32.7%) | 20 (36.4%) | 17 (30.9%) |

Descriptive statistics for the node level measures are presented in **Table 5**. Cows within all groups showed high between-individual variability (as shown by the standard deviation and range) in terms of initiating and receiving displacement and grooming. Furthermore, in the affiliative and agonistic networks, the OUT measures were slightly more variable than the IN measures. In the agonistic networks, the average degree and strength were similar in all groups with the same size. In the affiliative networks, these measures were smaller and they differed more between groups. In Group 2 during the autumn, both the average degree and average strength of grooming were higher than those in the spring despite the smaller group size.

**Table 5.** Descriptive statistics for node level measures in the displacement and grooming networks.

|                       | Displacement |       |     |     | Grooming |      |     |     |
|-----------------------|--------------|-------|-----|-----|----------|------|-----|-----|
|                       | Mean         | SD    | Min | Max | Mean     | SD   | Min | Max |
| <b>Group 1 Spring</b> |              |       |     |     |          |      |     |     |
| <b>(14 cows)</b>      |              |       |     |     |          |      |     |     |
| IN-Degree             | 10.57        | 1.50  | 8   | 13  | 5.57     | 2.10 | 0   | 9   |
| OUT-Degree            | 10.57        | 2.21  | 7   | 13  | 5.57     | 3.48 | 0   | 11  |
| IN-Strength           | 43.57        | 16.75 | 13  | 75  | 11.86    | 5.64 | 0   | 24  |
| OUT-Strength          | 43.57        | 22.11 | 15  | 93  | 11.86    | 9.49 | 0   | 30  |
| <b>Group 1 Autumn</b> |              |       |     |     |          |      |     |     |
| <b>(14 cows)</b>      |              |       |     |     |          |      |     |     |
| IN-Degree             | 9.57         | 1.55  | 7   | 12  | 6.71     | 2.09 | 3   | 10  |
| OUT-Degree            | 9.57         | 2.21  | 6   | 13  | 6.71     | 2.92 | 2   | 12  |
| IN-Strength           | 40.93        | 17.18 | 20  | 83  | 14.57    | 6.60 | 7   | 34  |
| OUT-Strength          | 40.93        | 18.76 | 22  | 80  | 14.57    | 9.82 | 2   | 34  |
| <b>Group 2 Spring</b> |              |       |     |     |          |      |     |     |
| <b>(14 cows)</b>      |              |       |     |     |          |      |     |     |
| IN-Degree             | 7.64         | 2.41  | 3   | 11  | 3.64     | 1.91 | 1   | 8   |
| OUT-Degree            | 7.64         | 3.10  | 1   | 12  | 3.64     | 1.95 | 0   | 7   |
| IN-Strength           | 42.14        | 20.59 | 4   | 78  | 7.64     | 7.11 | 2   | 30  |
| OUT-Strength          | 42.14        | 24.67 | 7   | 82  | 7.64     | 4.05 | 0   | 14  |
| <b>Group 2 Autumn</b> |              |       |     |     |          |      |     |     |
| <b>(11 cows)</b>      |              |       |     |     |          |      |     |     |
| IN-Degree             | 6.18         | 2.27  | 2   | 10  | 5.09     | 1.14 | 4   | 7   |
| OUT-Degree            | 6.18         | 3.25  | 0   | 10  | 5.09     | 2.34 | 1   | 10  |
| IN-Strength           | 31.09        | 13.78 | 7   | 48  | 13.64    | 7.58 | 5   | 34  |
| OUT-Strength          | 31.09        | 23.97 | 0   | 70  | 13.64    | 9    | 5   | 33  |

### 3.3 Indices of social behaviour

The DS, GI, and BI values were calculated for cows based on 3 days of data using the aggregated actor–receiver matrices. We investigated the relationships with BI calculated using the degree and strength measures but due to the high correlations ( $R_s$  range: 0.85–0.91), we only used the BI values based on the degree in subsequent analyses. We

compared the groups with respect to the relationships between the DS, GI, and BI values for cows (see S3 Fig. for details). In Group 1, the relationship patterns were similar during the spring and autumn, where cows with a high GI almost exclusively had positive BI values, but they were not necessarily high-ranking in terms of DS. In addition, some cows from the top and bottom of the dominance hierarchy had fairly similar BI values. In Group 2 during the spring, the observed patterns were similar to those in Group 1. By contrast, during the autumn, the total number of cows was smaller and the patterns were different, where a high GI often corresponded to negative BI values and there was a fairly good agreement between high DS rank and positive BI values.

### 3.4 Long-term stability of social behaviour

The correlations between the affiliative or agonistic interaction matrices during the spring and autumn were determined for the stable subgroups. In Subgroup 1, the  $R_{QAP}$  value was 0.291 ( $P = 0.052$ ) for affiliative matrices and 0.289 ( $P = 0.033$ ) for agonistic matrices. In Subgroup 2, we obtained a  $R_{QAP}$  value of 0.724 ( $P = 0.001$ ) for affiliative matrices and 0.395 ( $P < 0.001$ ) for agonistic matrices.

Regarding the temporal stability of DS, BI and GI, in Subgroup 1, all three indices had moderate to strong correlations between seasons, where this association was significant for GI (i.e., DS:  $R_S = 0.714$ ,  $P = 0.058$ ; BI:  $R_S = 0.667$ ,  $P = 0.083$ ; GI:  $R_S = 0.747$ ,  $P = 0.033$ ). In Subgroup 2, the association between spring and autumn was significant for all three indices (i.e., DS:  $R_S = 0.770$ ,  $P = 0.014$ ; BI:  $R_S = 0.855$ ,  $P = 0.004$ ; GI:  $R_S = 0.717$ ,  $P = 0.020$ ).

## 4. Discussion

### 4.1 Spatiotemporal distribution and stability of social interactions

According to the exploratory analysis, the frequency of displacements differed considerably between days, which was similar to the results obtained by Kondo and Hurnik (1990). The spatiotemporal distribution of displacements was similar each day, where most displacements occurred during the day-time and at the feeder, thereby supporting the findings of previous studies (DeVries et al., 2004; Val-Laillet et al., 2008). These observations indicate that automated measurement of replacements at the feeder (Huzzey et al., 2014) may provide sufficient information about the agonistic behavioural patterns within a group. However, the level of competition for lying stalls (Winckler et al.,

2015) or illness (Neave et al., 2018) could influence the reliability of these methods. In contrast to displacements, grooming was evenly distributed between the day-time and night-time, thereby highlighting the benefits of acquiring observations over 24 h for assessing affiliative relationships. Furthermore, we observed the majority of the grooming events at the lying stalls, whereas Val-Laillet et al. (2009) observed most grooming events at the feeder. It is possible that the cows in our study could not perform grooming at the feeder due to the isolating nature of the electronic bins.

Social behaviour in farm animal groups is a dynamic process (Estevez et al., 2007), and thus aggregating interactions over time may simplify some aspects of the social structure (Farine, 2018). However, representative snapshots could be used to assess positive welfare (Boissy et al., 2007) or social stress (Proudfoot and Habing, 2015). Previous studies of housing conditions often identified changes in the social interactions in small cattle groups by using observations obtained for 6–8 h per day over multiple days (Tresoldi et al., 2015; Améndola et al., 2016), or based on different observation times in different functional areas (Val-Laillet et al., 2009; Winckler et al., 2015). Thus, we investigated the most suitable time scale for acquiring reliable snapshots of affiliative and agonistic behaviours, and found that the daily interaction matrices had some correlations, but aggregating the interactions over 2 days improved the reliability. Furthermore, the changes in the numbers of observed affiliative and agonistic bonds as the number of days considered increased suggested that multiple days are needed to detect most existing relationships. These results indicate that continuous observations over 2–3 days may provide a reliable picture of affiliative and agonistic behavioural structures in small dairy cow groups in a barn.

#### **4.2 Analysis of affiliative and agonistic networks**

In general, we found more agonistic interactions than affiliative interactions, but the number of observed displacements and grooming events varied between seasons and groups. In both groups, displacements declined whereas grooming events increased from spring to autumn. In beef cattle, Nakanishi et al. (1993) obtained similar results where they also observed that the daily frequency of grooming increased when the number of agonistic interactions declined. The observed within-group changes contradict the theory

that grooming serves as a stress-reducing behaviour and that it increases as the level of social tension increases. We suggest that less competition might result in a more relaxed social environment and thus more grooming. However, according to our results it was not possible to distinguish whether the changes in the displacement and grooming frequencies were due to daily variation, group composition changes, or other factors.

There were no correlations between the affiliative and agonistic networks, thereby indicating that the two social behaviours followed different patterns within the groups. Our findings support previous studies of dairy cows (Val-Laillet et al., 2009) and heifers (Tresoldi et al., 2015), and they indicate that separate analyses of the two interaction types might not provide a complete picture of the social structure of a dairy cattle group. The agonistic networks were tightly knit in both groups and seasons. The groups showed differences in the network level measures, where displacement was mutual in most dyads in Group 1, whereas most displacement relationships were asymmetric in Group 2. These differences support the results obtained by Val-Laillet et al. (2008) who recorded displacements at the feeder in multiple dairy cattle groups. The between-group differences indicate that the individual characteristics of cows may affect the social behavioural patterns. Our groups were similar in terms of age, parity, and the number of days in milk for cows, and thus other individual characteristics, such as personality (Krause et al., 2010), might have influenced the social structure. The affiliative networks were less dense than the agonistic networks. Increased grooming during the autumn also resulted in social structure changes and the affiliative network was denser, especially in Group 2, where cows engaged in more grooming with more partners despite the smaller group size. In both groups and seasons, most grooming relationships were asymmetric, as shown in a previous study where grooming behaviour was not completely reciprocal (Val-Laillet et al., 2009). The asymmetric nature of grooming relationships raises the question of whether we can infer affiliative bonds based on undirected networks. Spatial proximity is thought to represent social bonds in dairy cattle (Gygax et al., 2010; Tresoldi et al., 2015; Boyland et al., 2016) and automatically collected proximity data have been used to create both directed and undirected affiliative networks in calves (Koene and Ipema, 2014; Chen et al., 2015). Our results suggest that directed edges may be more suitable for studying affiliative behaviour in dairy cattle because using undirected edges can mean the loss of

considerable amounts of information if most grooming is asymmetric. However, grooming and spatial proximity might represent different aspects of bonding (Dunbar and Shultz, 2010), so considering both may help to better understand the affiliative relationships within a herd. The node level measures of affiliative and agonistic networks showed considerable variability between individuals, which agrees with previous observations of grooming behaviour in beef cattle (Sato et al., 1993; Šárová et al., 2016) and dairy cattle (Wood, 1977). These measures might be affected by the individual characteristics of cows and their variability indicates that specific cows could have different roles in both social networks.

### **4.3 Indices of social behaviour**

In addition to the widely used DS value, we calculated GI values to represent the affiliative behavioural patterns. A similar index was used previously in brown capuchin monkeys (Parr et al., 1997), but it has not yet been applied in dairy cows to the best of our knowledge. DS and GI represent either agonistic or affiliative relationships, so we attempted to describe the complete social experience of a cow within the group using BI. We investigated the relationships between BI, DS, and GI, and compared the observed patterns between the groups in order to analyse whether dominance is related to an overall positive social experience in the group. In general, high dominance rank was not necessarily associated with high GI scores. Furthermore, BI did not have clear associations with the other indices. These results support the idea that the complete social experience of a cow is not accurately represented by dominance or grooming alone. Our results suggest that dominance may be more costly for some cows than others and that being at the bottom of the dominance hierarchy does not necessarily mean a negative social experience. To the best of our knowledge, the proposed BI measure is the first to combine affiliative and agonistic behaviours into a single comprehensive measure, and it might facilitate future investigations of how the welfare of individuals is related to their social environment.

### **4.4 Long-term stability of social behaviour**

In general, the affiliative and agonistic networks in the stable subgroups had associations between seasons. In Subgroup 2, both networks showed higher stability than those in Subgroup 1, probably due to the smaller change in the group composition in Group 2. Moreover, the presence or absence of specific cows potentially influenced the interactions within the stable subgroup, which might explain the low correlations between seasons, especially in Group 1. Our findings agree with previous observations of proximity-based networks in dynamic dairy cattle groups (Boyland et al., 2016) and they suggest that the consistency of social networks in dynamic groups may depend on the characteristics of the cows that are introduced or leave between observation periods (Krause et al., 2010; Boyland et al., 2016). The individual DS, GI, and BI scores for cows showed greater temporal consistency than the social networks within subgroups, and thus although the relationships within a network could change over time, the social behavioural characteristics of individuals represented by the indices were more robust. The consistency of dominance has been documented in previous studies (Wierenga, 1990; Bouissou et al., 2001) and the temporal stability determined in our study supports the suggestion that dominance might be considered a personality trait (Finkemeier et al., 2018). In contrast to dominance, little information is available on the group level stability of grooming (Wasilewski, 2003). The stability of GI in our study indicates that cows might consistently express affiliative behaviour even within a changing environment. Thus, individual cows may exhibit longer term stability in terms of their social behavioural characteristics, which might be important when considering the effect of the group composition on the welfare of individuals.

## 5. Conclusions

According to our exploratory analysis, we found that 3 days of observations provided a reliable snapshot of the affiliative and agonistic behavioural structures in small dairy cattle groups in a free stall barn. SNA based on 3 days of continuous observations of social interactions detected tightly connected agonistic networks with several mutual displacement relationships, whereas the affiliative networks were less dense and the majority of the grooming relationships were asymmetric, thereby highlighting the importance of directed networks for the analysis of social behaviour. The high variability of the node measures in all the networks obtained, i.e., high between-individual

variability in social behaviour, might be related to the individual personalities of cows. Furthermore, comparative analysis of dominance, grooming, and the proposed BI measure indicated that combining affiliative and agonistic behaviours into one measure could provide new insights into the overall social experience of cows. Finally, the stability of affiliative and agonistic behavioural patterns over 6 months indicates that cows exhibited consistent social behavioural characteristics, which supports the importance of the social environment for animal welfare.

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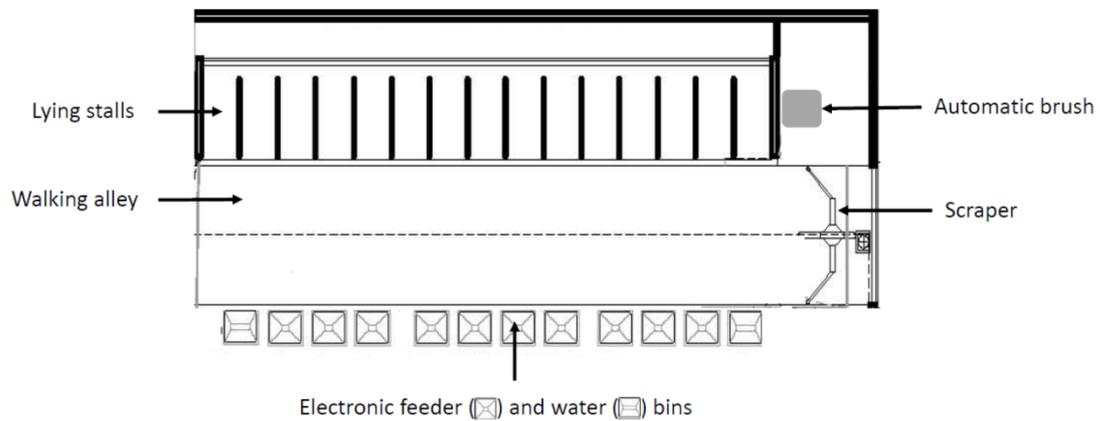
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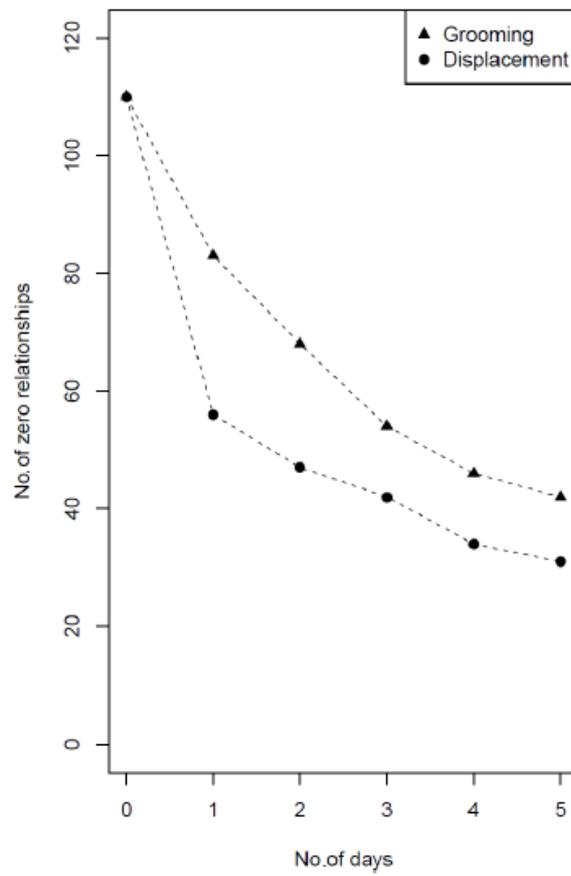
## Supporting information

**S1 Table.** Spatiotemporal distribution of displacement and grooming interactions over 5 days in Group 2.

| Zone                | Day 1       | Day 2       | Day 3      | Day 4       | Day 5       |
|---------------------|-------------|-------------|------------|-------------|-------------|
| <b>Displacement</b> |             |             |            |             |             |
| <b>Total</b>        | <b>146</b>  | <b>122</b>  | <b>74</b>  | <b>128</b>  | <b>211</b>  |
| Feeder              | 122 (83.6%) | 100 (82.0%) | 56 (75.7%) | 114 (89.1%) | 169 (80.1%) |
| Lying stalls        | 4 (2.7%)    | 9 (7.4%)    | 9 (12.2%)  | 8 (6.1%)    | 10 (4.7%)   |
| Walking alley       | 20 (13.7%)  | 13 (10.7%)  | 9 (12.2%)  | 6 (4.7%)    | 32 (15.2%)  |
| <b>Grooming</b>     |             |             |            |             |             |
| <b>Total</b>        | <b>52</b>   | <b>46</b>   | <b>52</b>  | <b>48</b>   | <b>43</b>   |
| Lying stalls        | 37 (71.2%)  | 26 (56.5%)  | 33 (63.5%) | 31 (64.6%)  | 34 (79.1%)  |
| Walking alley       | 15 (28.8%)  | 20 (43.5%)  | 19 (36.5%) | 17 (35.4%)  | 9 (20.9%)   |



**S1 Fig.** Group area in the barn



**S2 Fig.** Number of actor–receiver pairs without interactions (zero values in the matrix) as the number of days considered. Day 0 indicates the number of possible actor–receiver pairs in the group.

**S1 Document. Balance index**

The balance index (BI) is calculated with the following equation using social network measures:

$$BI = \frac{\frac{IN - Grooming + c}{IN - Displacement}}{\frac{OUT - Grooming + c}{OUT - Displacement}} = \frac{(IN - Grooming + c) * OUT - Displacement}{IN - Displacement * (OUT - Grooming + c)}$$

where IN-Grooming/IN-Displacement is either the number of grooming/displacement events received by a specific cow (calculated with IN-Strength) or the number of group members that groom/displace the specific cow (calculated with IN-Degree). OUT-Grooming/OUT-Displacement is determined in the same manner. Furthermore, *c* is a correction weight with a constant value (*c* = 0.005).

BI is transformed after calculating the ratio in order to obtain a symmetrical scale, as follows.

BI < 1 → -1/BI

BI = 1 → 0

BI > 1 → BI

We decided to add *c* to the grooming measures in order to differentiate between scenarios where IN and OUT ratio values were the same but there were differences in terms of the IN and OUT numbers for partners/interactions, as shown in the following example.

|                           | Case 1          | Case 2          | Case 3          | Case 4          |
|---------------------------|-----------------|-----------------|-----------------|-----------------|
| <i>IN – Grooming</i>      | 1 (+ <i>c</i> ) | 3 (+ <i>c</i> ) | 3 (+ <i>c</i> ) | 1 (+ <i>c</i> ) |
| <i>IN – Displacement</i>  | 3               | 1               | 3               | 1               |
| <i>OUT – Grooming</i>     | 1 (+ <i>c</i> ) | 3 (+ <i>c</i> ) | 1 (+ <i>c</i> ) | 3 (+ <i>c</i> ) |
| <i>OUT – Displacement</i> | 3               | 1               | 1               | 3               |
| <b>BI Original</b>        | <b>0</b>        | <b>0</b>        | <b>0</b>        | <b>0</b>        |
| <b>BI with <i>c</i></b>   | <b>0</b>        | <b>0</b>        | <b>-1.003</b>   | <b>1.003</b>    |

**BI range calculated with degree (number of partners):**

Assumption: Each cow is an actor at least once and a receiver of grooming as well as displacement (the slight effect of the constant *c* is neglected in this example).

*N* = number of animals in the group

- **Maximum** value of BI:

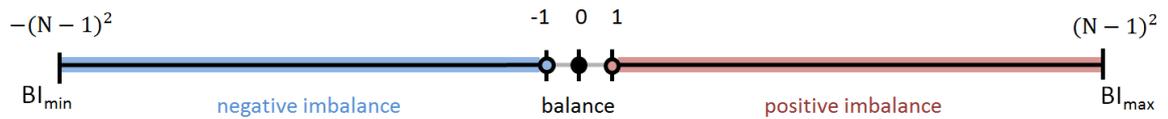
*IN – Grooming* and *OUT – Displacement* both reach the maximum of *N – 1* and *IN – Displacement* and *OUT – Grooming* both reach the minimum of 1

$$BI_{max} = (N-1)^2/1^2 = (N-1)^2$$

- **Minimum** value of BI:

*IN – Grooming* and *OUT – Displacement* both reach the minimum of 1 and *IN – Displacement* and *OUT – Grooming* both reach the maximum of *N – 1*

$$BI_{min} = -1/(1/(N-1)^2) = -(N-1)^2$$



### BI range calculated with strength (number of interactions):

This was calculated in a similar manner to the degree, but the range could be determined only after data collection because it depends on the total number of observed grooming ( $N_{groom}$ ) and displacement ( $N_{disp}$ ) events.

$$BI_{range} = \pm (N_{disp}-1) * (N_{groom}-1)$$

### Effect of zero values when calculating BI:

In order to calculate BI, a data set was preferred where each cow gave and received at least one grooming event and one displacement (no zero values in the ratios). However, it is possible that despite an adequate sampling effort a cow might never be an initiator or recipient of affiliative or agonistic interactions. In this case 0 values were replaced with 0.5 when calculating the ratio for a specific cow. By adding only a “half interaction” to a cow that had none, we avoided interfering with the network structure because adding one interaction would require choosing a partner for the cow to interact.

If an animal did not give/receive any grooming/displacement events, 0 in the ratio was replaced with 0.5. Thus the BI range changed as follows.

BI calculated with degree:

If there is only 1 zero value:  $BI_{max} = (N-1)^2/0.5*1 = (N-1)^2*2$  and  $BI_{min} = -(N-1)^2*2$

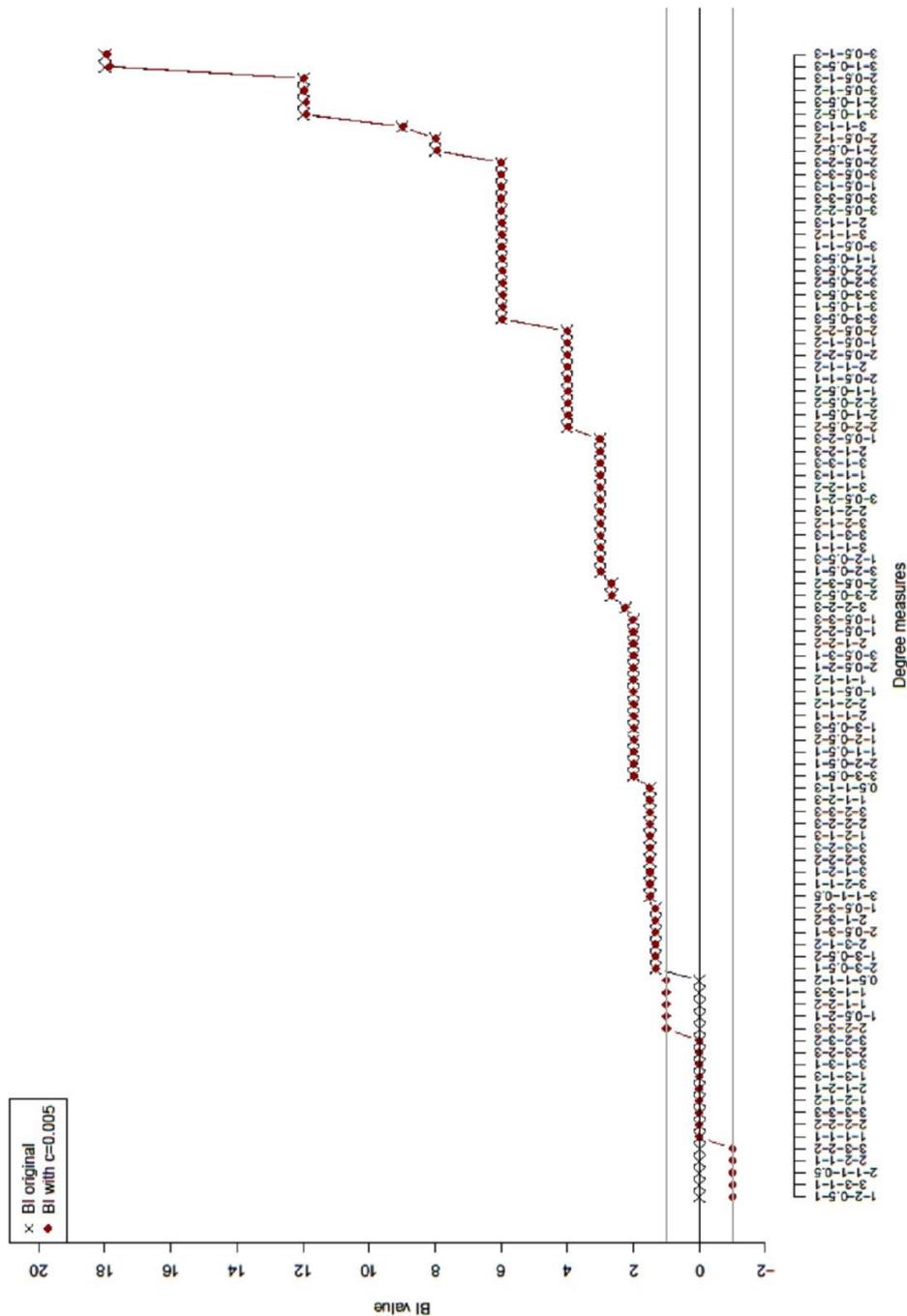
If there are more zero values:  $BI_{max} = (N-1)^2/0.5^2 = (N-1)^2*4$  and  $BI_{min} = -(N-1)^2*4$

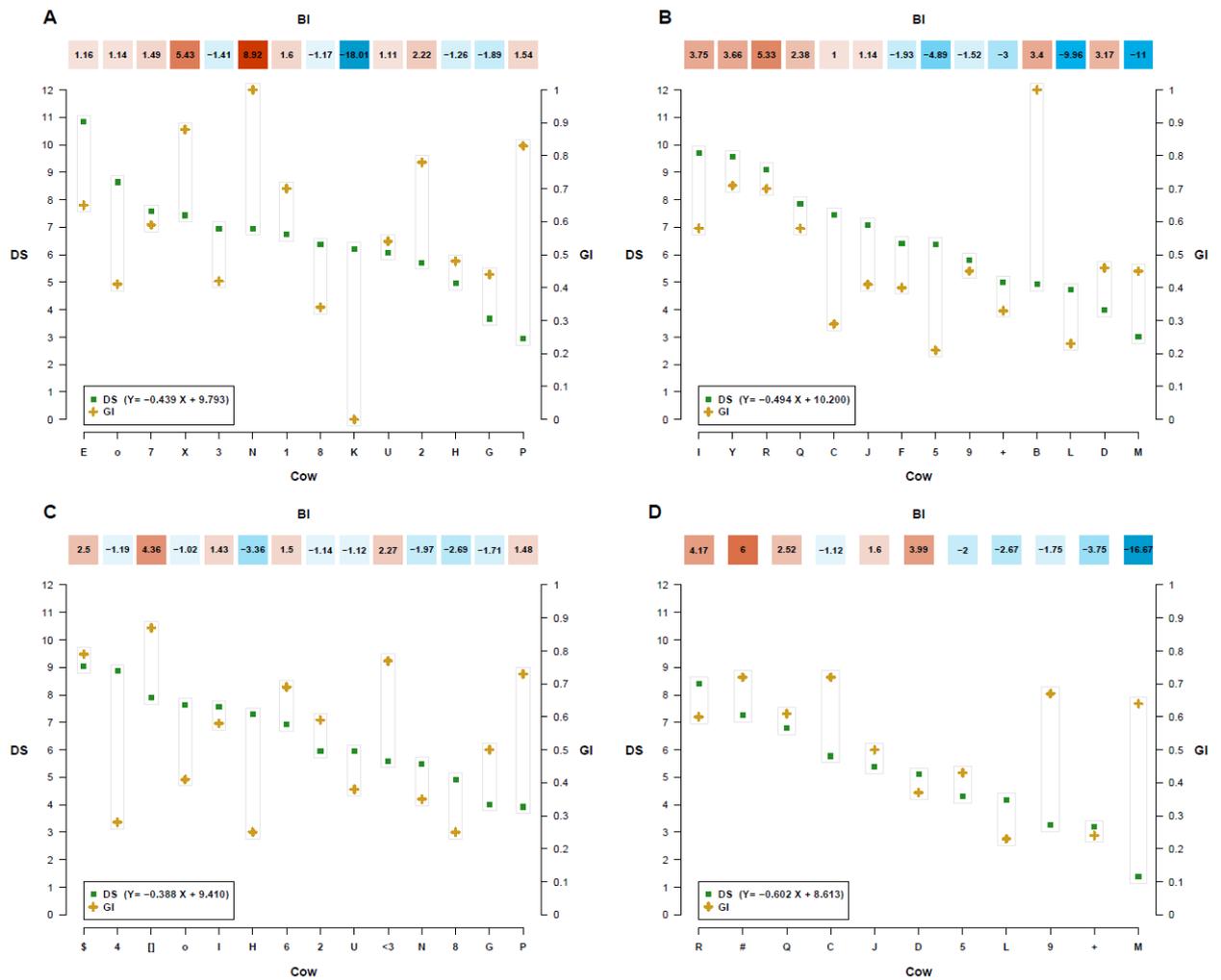
(The BI range calculated with strength changes in an analogous manner.)

**Example to illustrate the effects of the correction weight  $c$  and of replacing 0 values with 0.5 when calculating the BI ratio for cows that were not initiators or recipients of affiliative or agonistic interactions.**

In a group of four cows, we calculated the BI values corresponding to different degree measure combinations. In this example, we allowed one measure to be 0 and replaced it with 0.5. In the figure, we only show the degree measure combinations that resulted in positive or zero BI because negative BI values produced a symmetrical pattern.

The X-axis labels present the degree measures in the following order.  
*IN-Grooming – IN-Displacement – OUT-Grooming – OUT-Displacement*





**S3 Fig.** Relationships between the dominance score (DS), grooming index (GI) and balance index (BI) for cows in each group and season (A: Group 1 spring; B: Group 2 spring; C: Group 1 autumn; D: Group 2 autumn). Cows within each group are presented in descending dominance order. The equation describing the fitted line (steepness of the hierarchy) is provided in parentheses.

**DS:** Normalized David’s score where higher values indicate more dominant cows.

**GI:** Ratio of grooming received to all grooming involving a cow. Values above 0.5 indicate that more grooming was received than performed, whereas values below 0.5 indicate the opposite.

**BI:** Relationship between received and given grooming, and displacement of a cow. Positive values indicate that the incoming grooming to displacement partner ratio is higher than the outgoing ratio, whereas negative values indicate the opposite.

### **2.3. Automatic detection of agonistic behavior and dominance in dairy cows**

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Borbala Foris designed the study, analyzed the data, and wrote the manuscript with the support of and in agreement with her supervisor Dr. Nina Melzer and the co-authors of this manuscript.

## Automatic detection of agonistic behavior and dominance in dairy cows

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### ABSTRACT

Accurate assessments of social behavior and dominance relationships in cattle can be time consuming. We investigated if replacements at the feed bunk and water trough – one type of agonistic interaction – can be used to automatically assess dominance relationships. Our study set out 1) to validate a replacement detection algorithm using combined data from electronic feed and water bins, and 2) to investigate the applicability of this algorithm to identify dominance structures in free-stall-housed dairy cows. We used 4 groups of lactating cows kept in different group sizes (11 to 20 cows) located at 2 research facilities. In both facilities feed and water were provided via automated feeding systems. A trained observer recorded all agonistic interactions in the pen over multiple days using video. Data from the electronic feed and water bins for the same days were analyzed using an algorithm to detect replacements (i.e., visits where the ‘receiver’ was competitively replaced by the ‘actor’). Most agonistic interactions at the feed bunk were replacements. These replacements were associated with a brief interval between the time one cow left the bin and another took her place, although the optimal threshold to detect these replacements varied from 22 to 27 s between groups, independent of stocking density. The recall and precision of an algorithm based upon this threshold was high, comparable to trained human observers. Improved data preparation and filtering resulted in a > 20 % reduction in false positives and a 4.3 % increase in precision. The dominance hierarchy based upon algorithm-detected replacements reflected the dominance

hierarchy based on the total agonistic interactions observed in the pen; the Spearman's correlation coefficient between these hierarchies varied among the groups from 0.81 to 0.96. We conclude that data from electronic feed and water bins can accurately estimate agonistic behavior and dominance relationships among dairy cows.

**Key Words:** social competition, precision livestock farming, welfare

## INTRODUCTION

Automated methods are increasingly used to collect data on proximity and avoidance in cattle (Gygax et al., 2007; Boyland et al., 2013; Koene and Ipema, 2014) and these associations are sometimes used as a proxy for social interactions (Boyland et al., 2016). Detecting dyadic interactions could provide more specific information about social relationships (Foris et al., 2019). Existing work on social behavior in dairy cattle has relied on live observation or video (e.g., Wierenga, 1990; Val-Laillet et al., 2009; Tresoldi et al., 2015). Agonistic interactions occur mostly in areas where competition for resources is high, such as the feed bunk, drinker, lying stalls, mechanical brush, and automatic milking system (e.g., Val-Laillet et al., 2008a; Guzhva et al., 2016). Automated measures of attendance in these areas may also be used to estimate agonistic interactions (Guzhva et al., 2016, 2018; Huzzey et al., 2014; McDonald et al., 2019).

Previous work has assessed the use of an algorithm to detect agonistic replacements at the feed bunk using the data from an electronic feeding system (Huzzey et al., 2014; McDonald et al., 2019). A short time interval between visits by 2 different cows at the same bin was used to identify cases in which a 'receiver' cow was competitively replaced by an 'actor' cow. The optimal interval for identifying replacements was 26 s for feed bins (Huzzey et al., 2014) and 29 s for water bins (McDonald et al., 2019). Multiple studies have reported misidentification of cows and other technical issues (e.g., Chapinal et al., 2007; Ruuska et al., 2014; McDonald et al., 2019), but it is unclear how the performance of replacement detection algorithms are influenced by these issues. Moreover, the general applicability of a replacement detection algorithm to assess social relationships such as dominance has not been studied.

The objectives of the current study were to 1) evaluate the performance of a replacement detection algorithm using combined feed and water bin data, with a special

focus on investigating the reasons for false positive and false negative detections, 2) determine the efficacy of the algorithm for assessing the dominance structure within the group, and 3) test the robustness of the algorithm by applying it to different groups of dairy cows housed on different farms.

## MATERIALS AND METHODS

### *Animals and Housing*

We followed 4 groups of lactating Holstein cows (see **S1 Table** for age, parity, and DIM). Two groups of 20 cows were at the University of British Columbia Dairy Education and Research Centre in Agassiz, BC, Canada (UBC 1 and 2), housed in pens containing 24 lying stalls, 12 electronic feed bins, and 2 electronic water bins (see Neave et al., 2017 for a full description of housing conditions). Two other groups were at the Leibniz Institute for Farm Animal Biology in Dummerstorf, Germany (FBN 1: 14 cows, FBN 2: 11 cows), housed in pens containing 15 lying stalls, 10 electronic feed bins, and 2 electronic water bins (see Foris et al., 2019 for a full description of housing conditions). Thus, the groups differed in the stocking ratio of cows to feed bins, water bins, and lying stalls. All groups used Insentec feed and water bins (Roughage Intake Control System; Insentec, Marknessee, The Netherlands; validated by Chapinal et al., 2007 for intake and attendance measures) to provide ad libitum access to a TMR and water. To facilitate the identification of cows from video, cows were marked with unique alphanumeric symbols. Animals were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 2009) and the ASAB/ABS Guidelines for the Use of Animals in Research (Anonymous, 2016). Data collection was approved by the UBC Animal Ethics Committee (protocol A10-0163) and by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Western Pomerania, Germany (Reference number: 7221.3-2-033/15).

### *Video Recording and Analysis*

The 2 UBC groups were video recorded continuously for 2 consecutive days in the summer of 2013 using 4 Panasonic WVCW504SP video cameras (Panasonic Corp., Osaka, Japan) mounted approximately 6 m above the experimental pen. Cameras were connected to a digital video surveillance system (GeoVision, GeoVision Inc., Corona, CA,

USA). Red lights (100 W) were hung next to the cameras to facilitate video recording at night. The FBN groups were video recorded in the Autumn of 2016, over 3 consecutive days where each group was recorded with 2 cameras (Sony YC 3189, Sony Corp., Tokyo, Japan; including infrared light) and a digital recorder (EDR HD-2H14/4H4, EverFocus Electronics Corp., New Taipei City, Taiwan).

All videos were analyzed using Mangold Interact V15 (Mangold International GmbH, Arnstorf, Germany) following Foris et al. (2019). All agonistic interactions in the entire pen were identified continuously. We excluded milking time, which began when the first cow left the pen before milking and ended when the last cow entered the pen after milking, and times when management activities disturbed the cows, such as cleaning the lying stalls. Agonistic interactions were defined as: 1) Displacements at the bins (aggressive contact that resulted in the 'receiver' leaving the bin); replacements were those displacements where the 'actor' took occupancy of the bin within 60 s after the 'receiver' left; and, 2) Displacements at the lying stall or the walking alley (aggressive contact that resulted in the 'receiver' leaving the stall or taking a minimum of 2 steps in the walking alley).

### ***Replacement Detection Algorithm***

All data preparation and analyses were performed using R version 3.4.2 (R Core Team, 2017).

***Data Preparation.*** Data were downloaded from the electronic feed and water bins recording the start and end time of each visit and the identity of the cow. Data were combined for feed and water bins in each group and summarized by day (visits could not overlap days since all bins closed shortly before midnight and reopened just after midnight). A technical issue associated with the water bins in group UBC 1 during the first day resulted in these data not being used. As a first step, data were screened to exclude any cow identification numbers from cows not housed within the group. Also, any times from when the bins were in the closed position (i.e., '0' transponder entries) were removed. As a second step, we checked for technical errors by visually assessing the plausibility of visit durations using boxplots to identify outliers; feeder visits longer than 30 min and drinker visits longer than 10 min were individually validated using video observation. We also quantified bouts when a cow was registered at 2 bins simultaneously

and determined their cause based on video. As a third step, for each bin the time ordered data was used to determine the replacements by calculating the time difference between the exit time of one cow and the entry time of another; a replacement was recorded when this interval was less than or equal to a set threshold. Replacements were discarded when: 1) the replacement was recorded during milking times or other management activities that were excluded from the video analysis (**S2 Table**), or 2) the ‘actor’ was registered at another bin at the exit time of the ‘receiver’ (meaning that it was impossible that physical contact between the cows could have resulted in this replacement, the ‘actor’ occupied the bin after the ‘receiver’ left).

**Evaluating the Performance of the Algorithm.** First, we determined the proportion of agonistic interactions in the groups which can possibly be detected by the electronic bins. For this, we calculated the percentage of replacements from displacements at the bins and from all agonistic interactions in the pen. Replacements identified by the human observer were considered the gold standard to assess replacements identified by algorithm. True positives (TP) were replacements identified both the observer and the algorithm, false positives (FP) were replacements found only by the algorithm, and false negatives (FN) were replacements found only by the observer. True negatives were not interpretable in our analysis because video recorded replacement events were used as a gold standard (rather than all feeder visits regardless of whether a replacement occurred). Recall (also called sensitivity) was calculated to measure the quantity of replacements found, and precision was calculated to measure the quality of the revealed replacements:

$$Recall = \frac{TP}{TP + FN}$$

$$Precision = \frac{TP}{TP + FP}$$

To characterize the tradeoff between recall and precision in the different groups, we calculated both measures for thresholds ranging from 1 – 60 s. To identify the optimal threshold, we used the F-score (i.e., the harmonic mean of recall and precision; Saito and Rehmsmeier, 2015):

$$F\text{-score} = \frac{2 * Recall * Precision}{Recall + Precision}$$

For each group, the threshold associated with the maximum F-score was considered optimal and these thresholds were then used for further analysis.

We investigated the reasons for FN and FP in a more detailed manner. First, for TP events we assessed the association between replacement durations as determined by the algorithm versus the observer and found a very good agreement in each group (mean difference < 1s). Therefore, in the detailed analysis we excluded those FN events where the replacement duration determined by the observer was longer than the threshold of the algorithm. To provide a comparison between the performance of the algorithm and the observer, an independent trained observer reviewed the FN and FP events and decided whether a replacement happened. FN events (i.e., replacements detected only by the first observer) were categorized as 'replacement' if the bin did not detect the replacement (e.g., due to technical error) and categorized as 'no replacement' if the first observer made an error (i.e., misidentified the cow or coded a replacement instead of displacement). FP events (i.e., replacements detected only by the electronic bins) were categorized as 'no replacement' if there was no aggressive contact between the 'actor' and the 'receiver', and 'replacement' if a replacement happened but had not been detected by the first observer. This validated data set was then used as a gold standard to recalculate recall and precision and to compare these between the algorithm, and the first video observer. Furthermore, we assessed the number of FP replacements which were eliminated by our introduced filtering step (i.e., controlling for the presence of the 'actor' at other bins, see above) and separately assessed replacements for when data from only feed bins or water bins were used.

### ***Dominance Assessment***

We investigated if the algorithm could be used to assess the social hierarchy within the groups, calculating dominance scores (DS) using the normalized David's score (de Vries et al., 2006) as implemented in the R package *steepness* (Leiva and de Vries, 2014). We controlled for the ratio of sufficient interactions to cows (i.e., at least 10 times as many interactions as cows in the group; Sánchez-Tójar et al., 2018). We used 4 different data sets in a stepwise approach to obtain hierarchies: 1) Replacements revealed by the algorithm, termed *Bin DS*, 2) replacements at the bins identified by the observer, 3) displacements at the bins identified by the observer, and 4) displacements at the bins and

in other zones as identified by the observer, termed as *Complete DS*. For each group, we calculated the associations between these dominance hierarchies using Spearman's rank correlation ( $r_s$ ). In a detailed investigation we compared *Bin DS* and *Complete DS* on the group level based on their steepness (measuring the degree of individual differences in winning dominance encounters; de Vries et al., 2006), and investigated the agreement in individual David's scores between *Bin DS* and *Complete DS*.

## RESULTS

### Video Analysis

The numbers of replacements and additional displacements are presented in **Table 1**. In all groups, the majority of agonistic interactions occurred at the feed bunk, more than two thirds of which were replacements. These replacements represented roughly the half of all agonistic interactions in the pen.

**Table 1.** Number of video observed replacements<sup>1</sup> and additional displacements<sup>2</sup> at the electronic bins, lying stalls and in the walking alley in each group. The proportion of replacements from agonistic interactions at the bins or in the pen is also shown.

| Group | Replacements | Additional displacements |              |               | Proportion of replacements |               |
|-------|--------------|--------------------------|--------------|---------------|----------------------------|---------------|
|       |              | Bins                     | Lying stalls | Walking alley | Agonistic at bins          | All agonistic |
| FBN 1 | 232          | 105                      | 68           | 159           | 0.69                       | 0.41          |
| FBN 2 | 196          | 83                       | 22           | 42            | 0.70                       | 0.57          |
| UBC 1 | 257          | 118                      | 16           | 49            | 0.69                       | 0.58          |
| UBC 2 | 357          | 165                      | 23           | 27            | 0.68                       | 0.62          |

1: Aggressive contact which results in the 'receiver' leaving the bin and the 'actor' is the first cow taking occupancy of the same bin within 60s.

2: Additional displacement at the bins: Aggressive contact which results in the 'receiver' leaving the bin but the 'actor' is not the first cow taking occupancy of the same bin within 60s.

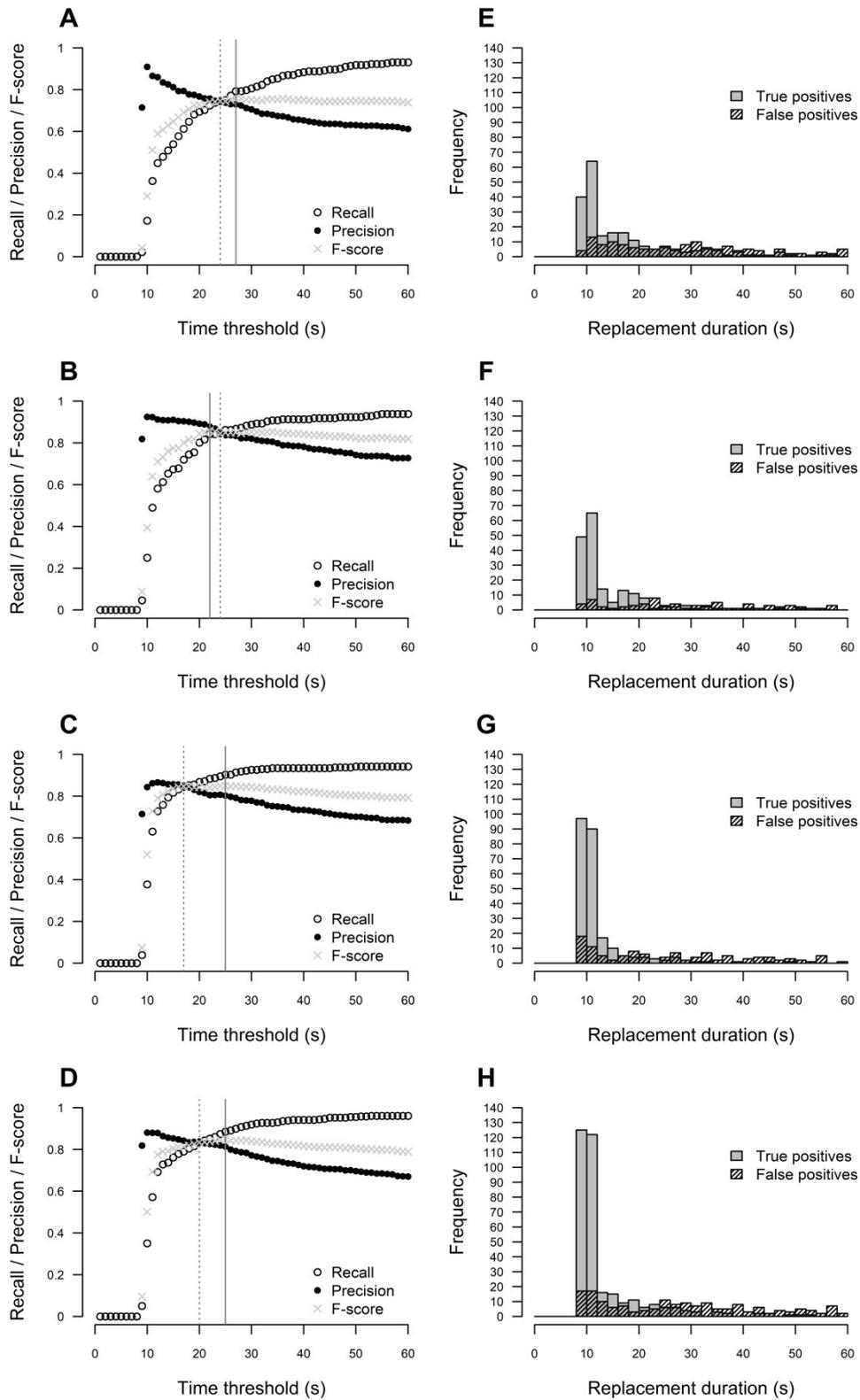
Additional displacement in other zones: Aggressive contact, the 'receiver' leaves the lying stall or takes minimum 2 steps in the walking alley.

### Replacement Detection Algorithm

The video validation showed (see **S1 Figure** for the box plots of bout durations) that long bouts were not caused by a technical error, but rather by cows truly occupying the bin for a long time. Cows were detected simultaneously at 2 bins in less than 1 % of

bin visits (**S2 Table**); all these events were caused by one cow replacing another without the bin registering the change, rather than by a bin staying open after a cow left.

The tradeoff between the quantity and quality of replacements revealed by the algorithm with increasing threshold is shown in **Figure 1 (A – D)**. Groups varied in the optimal threshold revealed by the maximum F-score (mean  $\pm$  SD:  $24.8 \pm 2.06$  s), and in the threshold where recall and precision were roughly equal (mean  $\pm$  SD:  $21.3 \pm 3.40$  s). In all groups recall and precision values were high (around 0.8) across thresholds ranging from 15 to 30 s. Few intervals longer than 30 s were replacements and most replacements were 9 to 12 s (**Figure 1 E – H**). Accordingly, thresholds  $> 30$  s were associated with only a slight increase in recall and a decline in precision (i.e., the increase in TP was associated with a higher amount of FP). Thresholds corresponding to 9 – 12 s were associated with high precision (i.e., almost all detected replacements were true), but they were worse in recall (i.e., many replacements were missed).



**Figure 1.** Recall, precision, and F-scores of the replacement detection algorithm for different thresholds in the groups (A: FBN 1, B: FBN 2, C: UBC 1, D: UBC 2). Gray lines show the threshold with the maximum F-score, dashed gray lines show the point where recall and precision are roughly equal. The distribution for the duration of replacements detected by the electronic bins (E: FBN 1, F: FBN 2, G: UBC 1, H: UBC 2) is also shown.

We compared the performance of the algorithm with the performance of the video observer using independent validation of FN and FP replacements. **Table 2** shows the number of replacements found by the observer and the algorithm, and the decision (i.e., replacement or no replacement) of the independent observer for replacements originally categorized as FN or FP. Based on this information as a gold standard, we calculated similarly high recall values for the algorithm and for the first observer; the precision of the algorithm was high (around 0.80), but lower compared to the precision of the observer.

**Table 2.** Evaluation of replacements detected at the electronic bins, comparison of recall and precision of the first video observer and the algorithm. In parentheses the corresponding values without using the additional filtering step to control for the presence of the ‘actor’ at other bins are shown

| Group | Threshold (s) | True positives | False positives |    | False negatives |    | Recall         |          | Precision      |          |
|-------|---------------|----------------|-----------------|----|-----------------|----|----------------|----------|----------------|----------|
|       |               |                | -               | +  | -               | +  | Bins           | Observer | Bins           | Observer |
| FBN 1 | 27            | 184<br>(185)   | 53<br>(68)      | 15 | 10<br>(2)       | 3  | 0.99<br>(0.99) | 0.93     | 0.79<br>(0.75) | 0.95     |
| FBN 2 | 22            | 165            | 20<br>(26)      | 3  | 2               | 8  | 0.96           | 0.98     | 0.89<br>(0.87) | 0.99     |
| UBC 1 | 25            | 232<br>(234)   | 39<br>(58)      | 18 | 7<br>(5)        | 7  | 0.97<br>(0.98) | 0.93     | 0.87<br>(0.81) | 0.97     |
| UBC 2 | 25            | 316            | 48<br>(71)      | 24 | 0               | 13 | 0.96           | 0.93     | 0.88<br>(0.83) | 1.00     |

Threshold: optimal threshold determined by the maximum F-score.

True positives: Replacements found by both the algorithm and the first observer.

False positives: Replacements found only by the algorithm using the optimal threshold.

False negatives: Replacements found only by the first observer, only replacements not longer than the optimal threshold are considered.

+: Replacement according to the independent observer.

- : No replacement according to the independent observer.

The filtering decreased the number of false detections (cf., **Table 2**). The number of FP decreased by an average of 27.6 % resulting in a 4.3 % increase in precision. Only a slight decrease in recall was observed in 2 groups due to filtering some replacements where the bin that was previously occupied by the ‘actor’ was still open when the ‘receiver’ left the bin.

The contribution of feed and water bins to detecting replacements is shown in **Table 3**. In all groups, the water bins recorded only a small proportion of all replacements. In groups where water bin data was used for all days, on average 4.3 % of TP, 11 % of FP, and 26.4 % of FN replacements were associated with water bins.

**Table 3.** Number of replacements detected by feed and water bins using the optimal threshold in each group. Replacements longer than the optimal threshold are excluded from false negatives (replacements found only by the video observer).

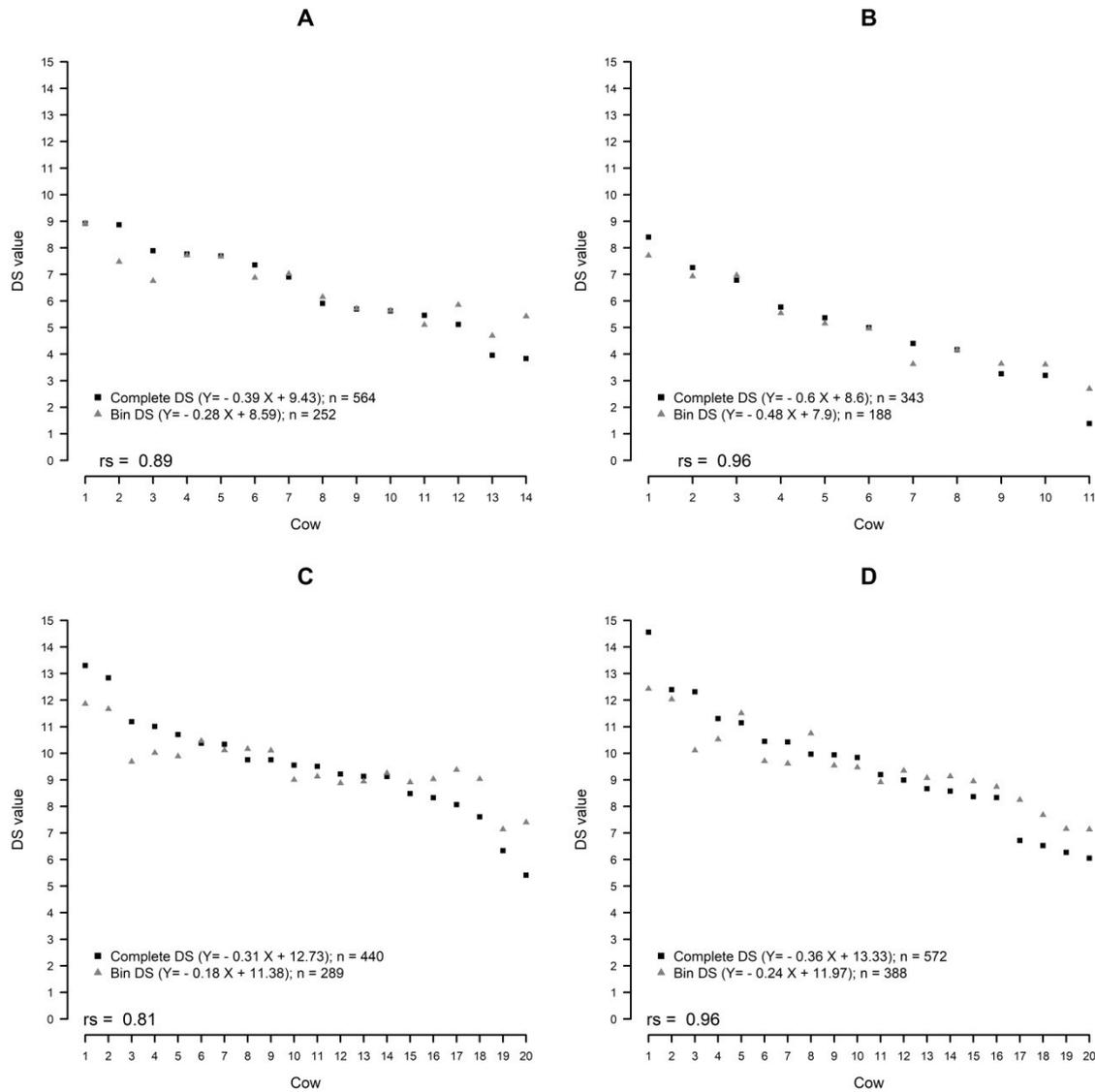
| Group | Threshold (s) | True positives |       | False positives |       | False negatives |       |
|-------|---------------|----------------|-------|-----------------|-------|-----------------|-------|
|       |               | Feeder         | Water | Feeder          | Water | Feeder          | Water |
| FBN 1 | 27            | 176            | 8     | 65              | 3     | 10              | 3     |
| FBN 2 | 22            | 164            | 1     | 23              | 0     | 9               | 1     |
| UBC 1 | 25            | 224            | 8     | 55              | 2     | 10              | 4     |
| UBC 2 | 25            | 291            | 25    | 56              | 16    | 7               | 6     |

### **Dominance Assessment**

In all groups, there was a strong association between the 4 different dominance hierarchies (**Table 4**; all  $r_s > 0.80$ ). Focusing on individual cows, we investigated if the algorithm can predict their *Complete DS* values. **Figure 2** provides evidence that electronic bins performed well in estimating dominance status. However, the algorithm overestimated DS for cows that were lower in the hierarchy and underestimated it for cows at the top of the hierarchy. In other words the algorithm generally resulted in flatter hierarchies (i.e., less difference in DS between the most and least dominant cow).

**Table 4.** Spearman's rank correlation coefficients between dominance hierarchies in 4 groups (FBN 1-2, UBC 1-2), calculated based on A: Replacements revealed by the algorithm using the optimal threshold; B: Observer replacements at the bins; C: Observer displacements at the bins; D: Observer displacements at the bins and in other zones

|              |   |      |      |      |
|--------------|---|------|------|------|
| <b>FBN 1</b> | A | B    | C    | D    |
| A            | 1 | 0.92 | 0.93 | 0.89 |
| B            |   | 1    | 0.98 | 0.87 |
| C            |   |      | 1    | 0.90 |
| D            |   |      |      | 1    |
| <b>FBN 2</b> | A | B    | C    | D    |
| A            | 1 | 0.97 | 0.96 | 0.96 |
| B            |   | 1    | 0.99 | 0.99 |
| C            |   |      | 1    | 1    |
| D            |   |      |      | 1    |
| <b>UBC 1</b> | A | B    | C    | D    |
| A            | 1 | 0.85 | 0.81 | 0.81 |
| B            |   | 1    | 0.96 | 0.95 |
| C            |   |      | 1    | 0.97 |
| D            |   |      |      | 1    |
| <b>UBC 2</b> | A | B    | C    | D    |
| A            | 1 | 0.97 | 0.96 | 0.96 |
| B            |   | 1    | 0.97 | 0.98 |
| C            |   |      | 1    | 0.99 |
| D            |   |      |      | 1    |



**Figure 2.** Dominance scores (DS: Normalized David’s score) of cows based on all observed agonistic interactions in the pen (Complete DS) and based on replacements detected by the algorithm using the optimal threshold (Bin DS) in the groups (A: FBN 1, B: FBN 2, C: UBC 1, D: UBC 2). Cows are presented in descending Complete DS order. For both dominance hierarchies the equations describing the fitted line are provided in parentheses. Number of interactions ( $n$ ) used for calculating DS values and Spearman’s rank correlation coefficients ( $r_s$ ) between Complete DS and Bin DS values of cows are also presented.

## DISCUSSION

We evaluated an algorithm based on combined electronic feed and water bin data to detect replacements at the feed bunk and automatically estimate the dominance hierarchy in groups.

### ***Video Analysis***

We used 2 to 3 days of continuous video to assess agonistic behavior within groups of lactating dairy cows. Previous work showed that this period provides a reasonable snapshot of the agonistic behavioral structure within a group (Foris et al., 2019). Milking times were determined based on video observation in this study, but the electronic feeder system combined with tri-axial accelerometers allow for the automated detection of times where cows are away (Thompson et al., 2017). Regarding the spatial distribution of agonistic interactions, our results indicate that if cows are not overstocked for lying stalls most social competition happens at the feed bunk, supporting previous findings (Val-Laillet et al., 2008a). To our knowledge, this study is the first to assess the proportion of replacements from all agonistic interactions in dairy cows. In all groups, replacements represented the majority of agonistic interactions at the feed bunk, and a high percentage of all agonistic interactions in the pen. This finding supports the use of electronic bins for the automatic recording of social competition.

### ***Replacement Detection Algorithm***

When using automated data it is important to control for systematic errors. We included quality checks in the data preparation procedure. Assessing outliers at the bins may detect if a bin does not close when a cow leaves and could serve as an indicator for mechanical or electronic problems. The simultaneous detection of a cow at 2 bins can be the result of the above-mentioned issue, or it can occur if a cow replaces another in a way that the bin does not detect (termed 'stealing' by Ruuska et al., 2014). The latter issue was marginal in our data, but such data checks may be of more value for long-term assessments.

To assess the use of electronic bins for monitoring social competition, we investigated how the algorithm threshold influenced the quality and quantity of replacements detected, and how these vary in different facilities and in groups with

varying stocking densities. We assessed replacements by video only when an agonistic contact from the 'actor' resulted in the occupation of that bin within 60 s. Our results confirmed that this limit was appropriate as the vast majority of replacements took place within 30 s (**Figure 1**). Based on the tradeoff between recall and precision we suggest using thresholds between 20 to 30 s when applying the algorithm in groups under similar housing conditions. In this range F-scores were high, but lower thresholds were often associated with much lower recall and higher thresholds corresponded to slightly lower F-scores. Previous studies examined data from feed bins (Huzzey et al., 2014) or water bins (McDonald et al., 2019) only, but in the current study we used these measured in combination. We observed too few replacements at the water bins to assess the data from these two sources separately. The optimal thresholds for feed (26 s) and water bins (29 s) identified in these earlier studies correspond well with the range identified here. Despite the small proportion of replacements at water bins observed in our study, we encourage combining feed and water bin data whenever possible; data from drinkers may be especially helpful during periods of heat stress. In addition, the data from the water bins can contribute to filtering FP replacements. Our filtering step increased the precision of the algorithm in all observed groups. The plausibility check of replacements by controlling if the 'actor' was detected at another location when the 'receiver' left the bin appears to be a promising approach for further improvements. Combining this algorithm with data from other sensors including accelerometers (Ledgerwood et al., 2010) and location tracking (Pastell et al., 2018) could improve performance.

Video observation is commonly used as the gold standard to evaluate behavioral detection methods. However, human observers are also prone to errors. We analyzed discrepancies between the observer and the algorithm. Recall was slightly lower for the observer compared to the algorithm in 3 groups (**Table 2**), indicating that the observer might miss more replacements than the algorithm. The precision was higher for the observer in all groups, because the algorithm detected a replacement even if a cow occupied a bin without any agonistic contact with the previous cow. However, the precision of the algorithm was generally high, confirming that in most cases short time intervals between cows at the same bin were associated with agonistic interactions. Our results indicate that the algorithm is comparable to human video observation in detecting

replacements at the feed bunk. Multiple studies have used this replacement detection algorithm with feed bin data (e.g., Sepúlveda-Varas et al., 2016; Crossley et al., 2017; Jensen and Proudfoot, 2017). The use of the algorithm facilitates long-term assessments for large groups that would be impractical using human observers, and more generally facilitates the inclusion of agonistic behaviors in future work.

### ***Dominance Assessment***

Studies of dominance in dairy cattle have a long history (e.g., Schein and Fohrman, 1955) and have questioned if patterns of social competition correspond to classical properties of dominance (Wierenga, 1990; Val-Laillet et al., 2008b, 2009). On commercial dairy farms, group composition is frequently changed according to the physiological status and nutritional needs of cows, affecting social behavior and production (e.g., von Keyserlingk et al., 2008; Schirmann et al., 2011). Long-term data collection on agonistic interactions may improve our understanding of dominance in dynamic groups, but it is not feasible using traditional observation methods. Our second objective was to assess if the algorithm can also be used to assess the dominance structure. The algorithm-based dominance showed very good agreement with dominance based on all observed agonistic interactions at the feed bunk, indicating that automated replacements can be used to assess social hierarchy at the feed bunk. These results also agree with previous findings showing that the success index of focal cows was highly correlated between automatically detected replacements and video recorded displacements at the feed bunk (Huzzey et al., 2014). However, different resources in the pen may be associated with different hierarchies according to the individual motivation of cows to gain access. Previous work found a low positive correlation between the competitive success of cows at the feed bunk and at the lying stalls (Val-Laillet et al., 2008a). In the current study, cows were overstocked for feed and water bins, but understocked for lying stalls in all groups, and the number of agonistic interactions was low in areas other than the feed bunk. Indeed, we recorded too few displacements at the lying stalls to meet the minimum for dominance calculation (cf., Sánchez-Tójar et al., 2018), and so could not determine if the competition at the feed bunk and at the lying stalls follow different patterns. Comparison of *Bin DS* and *Complete DS* suggests that differences in competitiveness of cows are less pronounced at the feed bunk (i.e., a subordinate cow may replace a dominant, perhaps

according to individual differences in satiety). Other work has shown that many bi-directional displacement relationships are observed at the feeder (Val-Laillet et al., 2008b). Despite the potential variation in hierarchies at different locations, high correlations between *Bin DS* and *Complete DS* show that the algorithm can reasonably estimate the dominance status of cows, at least under housing conditions similar to those in this study. The use of electronic bins for automatic dominance assessment may help understand associations between the social environment of cows and other factors such as health or productivity.

### CONCLUSIONS

Combined electronic feed and water bin data was used to detect agonistic replacements at the feed bunk in 4 groups of cows housed at different densities and in different facilities. We found that a 20 to 30 s interval between visits by 2 different cows at the same bin was optimal to identify replacements. The algorithm performed well in terms of quality and quantity of replacements revealed, showing similar reliability to human observations of video. The algorithm can also be used to assess dominance structure, facilitating work on social relationships in dairy cows.

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## APPENDIX

**S1 Table.** Number of cows, stocking density, and days analyzed within each group. For the age, parity, DIM, and feeding bout duration of cows, the mean  $\pm$  SD and in parentheses the range is provided.

| Group | No. of cows | Stocking density for lying stalls | Stocking density for feed bins | Stocking density for water bins | No. of observation days | Age (years)                   | Parity               | DIM                         |
|-------|-------------|-----------------------------------|--------------------------------|---------------------------------|-------------------------|-------------------------------|----------------------|-----------------------------|
| FBN 1 | 14          | 0.93                              | 1.4                            | 7                               | 3                       | 4.8 $\pm$ 0.9<br>(2.9 – 6.0)  | 2 $\pm$ 1<br>(1 – 4) | 241 $\pm$ 153<br>(4 – 573)  |
| FBN 2 | 11          | 0.73                              | 1.1                            | 5.5                             | 3                       | 4.9 $\pm$ 0.7<br>(3.0 – 5.5)  | 3 $\pm$ 1<br>(1 – 3) | 191 $\pm$ 103<br>(44 – 357) |
| UBC 1 | 20          | 0.83                              | 1.67                           | 10                              | 2                       | 4.1 $\pm$ 2.2<br>(2.0 – 10.0) | 3 $\pm$ 2<br>(1 – 7) | 18 $\pm$ 10<br>(5 – 32)     |
| UBC 2 | 20          | 0.83                              | 1.67                           | 10                              | 2                       | 3.8 $\pm$ 1.8<br>(2.0 – 10.0) | 3 $\pm$ 1<br>(1 – 7) | 20 $\pm$ 11<br>(5 – 39)     |

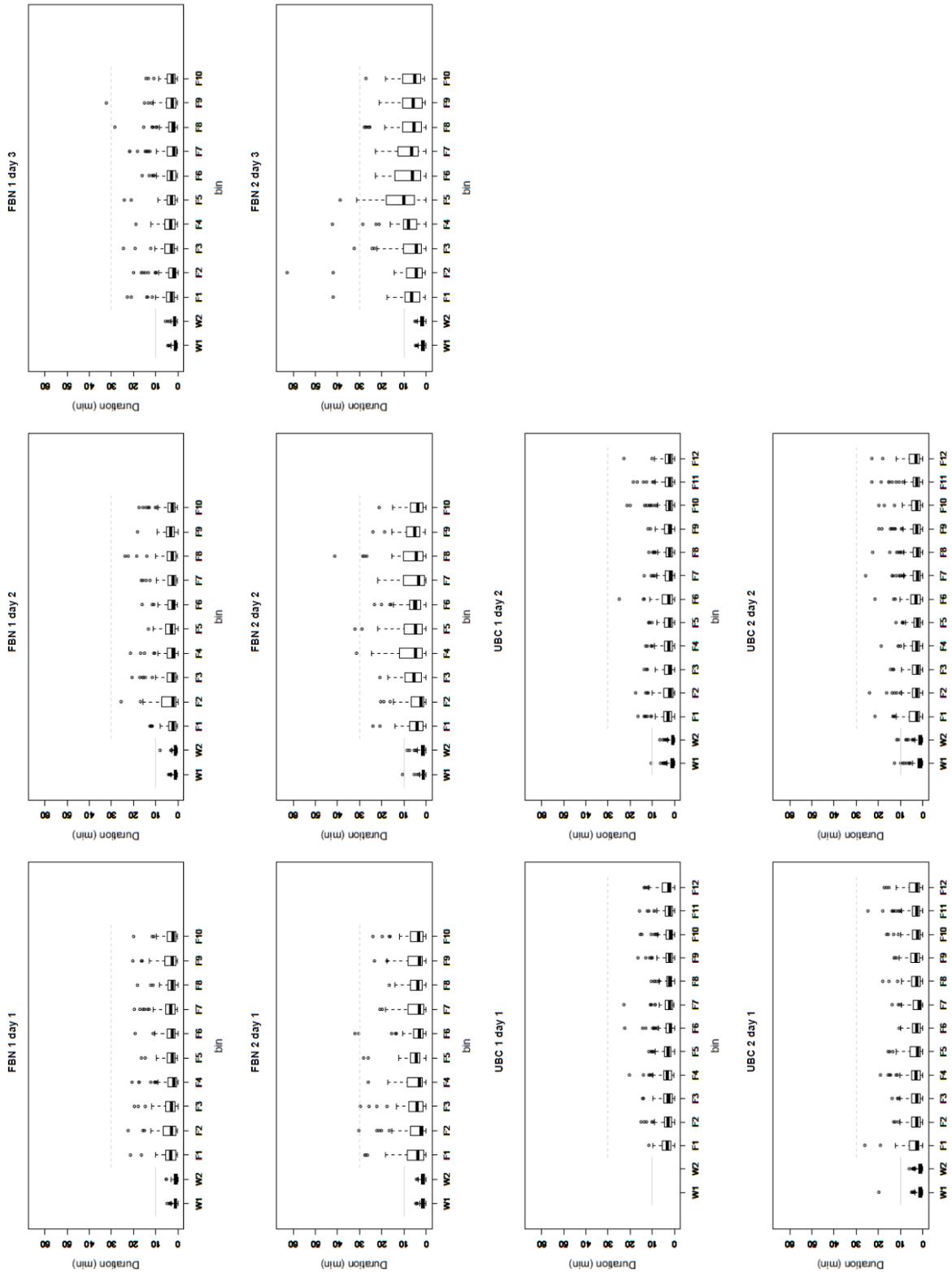
**S2 Table.** Number of daily feeding and drinking bouts; maximum (Max), mean, and SD of bout durations in the groups (FBN 1-2, UBC 1-2). The number and percentage of bouts when a cow was registered at 2 bins simultaneously and the time excluded from analyses is also presented.

| Group     | Cow at two bins <sup>1</sup> |            | Feed bins   |              |                    |                     | Water bins        |              |                    |                     | Time excluded <sup>2</sup> (min) |                   |
|-----------|------------------------------|------------|-------------|--------------|--------------------|---------------------|-------------------|--------------|--------------------|---------------------|----------------------------------|-------------------|
|           | No. of bouts                 | % of bouts | Total bouts | No. of bouts | Max duration (min) | Mean duration (min) | SD duration (min) | No. of bouts | Max duration (min) | Mean duration (min) |                                  | SD duration (min) |
| FBN1 day1 | 1                            | 0.114      | 876         | 709          | 22.383             | 3.759               | 3.827             | 167          | 5.300              | 1.206               | 0.897                            | 76                |
| FBN1 day2 | 1                            | 0.105      | 953         | 776          | 25.633             | 3.560               | 3.683             | 177          | 7.867              | 1.369               | 0.933                            | 79                |
| FBN1 day3 | 1                            | 0.110      | 910         | 741          | 32.333             | 3.801               | 4.045             | 169          | 5.467              | 1.426               | 0.926                            | 82                |
| FBN2 day1 | 5                            | 0.778      | 643         | 529          | 31.933             | 5.431               | 5.732             | 114          | 4.533              | 1.569               | 0.951                            | 76                |
| FBN2 day2 | 1                            | 0.177      | 566         | 448          | 41.067             | 6.261               | 6.239             | 118          | 10.633             | 1.732               | 1.589                            | 74                |
| FBN2 day3 | 1                            | 0.236      | 424         | 308          | 62.683             | 8.306               | 8.277             | 116          | 4.850              | 1.733               | 1.055                            | 75                |
| UBC1 day1 | 0                            | 0          | 1577        | 1197         | 22.633             | 3.199               | 2.933             | 380          | 19.567             | 1.479               | 2.072                            | 257               |
| UBC1 day2 | 0                            | 0          | 1579        | 1258         | 24.983             | 3.117               | 3.074             | 321          | 10.317             | 1.208               | 1.222                            | 309               |
| UBC2 day1 | 0                            | 0          | 1581        | 1262         | 26.167             | 3.520               | 3.261             | 319          | 19.683             | 1.312               | 1.506                            | 162               |
| UBC2 day2 | 0                            | 0          | 1680        | 1246         | 25.667             | 3.563               | 3.445             | 434          | 12.817             | 1.554               | 1.671                            | 311               |

1: Bouts where a cow was registered at two bins simultaneously.

2: Times excluded from analysis due to management activities such as cleaning the lying stalls or milking

**S1 Figure.** Daily durations of bouts for water bins (W1-W2) and feed bins (F1-F12) in the groups (FBN 1-2, UBC 1-2). Gray lines indicate the thresholds above which bouts were video validated.



### 3. General discussion

In this chapter, I discuss the novel approaches and methods for the assessment of individual personality and group social structure presented in this thesis, with an additional focus on possible implications for dairy cattle welfare. The key findings of the first study were that in contrast to the one-dimensional interpretation of temperament multiple personality traits exist in adult dairy cows, whereby aspects of these traits were consistent, whether measured in the group or in individual tests. These results are considered in relation with the knowledge about the social environment obtained in the second study. Briefly, the second study investigated affiliative and agonistic behavior on the individual- and group-level and revealed that they exhibit temporal stability but follow different patterns. These findings are discussed with a special focus on personality-trait-like characteristics of social network measures. The third study explored how the gained knowledge about the spatiotemporal distribution of social interactions can be used in automatic interaction detection methods to take a step towards the practical use of the findings. Finally, this section describes the possible ways how the findings of this thesis can be transferred to practice to improve the welfare of dairy cows.

#### 3.1. Personality in dairy cows

##### 3.1.1. Assessment individually

Even though the relevance of consistent individual behavioral variation in dairy cattle has been realized (Haskell et al., 2014), little knowledge exists on the structure and stability of personality traits in adult cows. Also, it has not been investigated prior to this work if a simple behavioral test performed in the social group could be used to replace the time-consuming testing of single animals. One aim of the first study was to apply a multi-test multi-trait approach to reveal the structure and temporal stability of personality in adult lactating cows. Here a combination of standardized individual behavioral tests (i.e., novel arena, novel object, novel human) was used, as it was previously found to be a reliable approach to assess behavioral variation in cattle (e.g., MacKay et al., 2014). However, the reliability of the obtained behavioral parameters can depend on factors such as object used, test duration or health status of tested animals (Meagher et al., 2016). Therefore,

to better represent personality traits, aggregated measures from tests in different contexts instead of a single measure arising from each test may be used (Lecorps et al., 2018b). In the first study, we reduced the number of behavioral parameters used according to the sample size (cf., section 1.3.4.) to ensure the robustness of the statistical analysis. From each behavioral test only those parameters were used in the PCA, which reflected the reaction of the cow to novelty, in order to avoid possible bias. Accordingly, from the novel arena test, parameters associated with locomotion and exploratory behaviors were used. Other studies often assessed these behaviors during the novel object or novel human test following the novel arena test (e.g., Graunke et al., 2013). However, with time cows show less movement and exploration in novelty tests performed in social isolation (Müller and Schrader, 2005b; MacKay et al., 2014; Mandel et al., 2019), probably due to the habituation to the test arena. Therefore, using these parameters from all three tests (or aggregating them) probably would have biased the results of the study in this experimental setup. Altogether, it seems beneficial to use these behavioral parameters only from the first test, or apply tests with more time in-between and calculate averages for further analysis (Lecorps et al., 2018b). From the second and third part of the individual test, latency to contact the object/human was excluded. The reason is that the object/human was presented afterwards the cow was already in the arena, but the test only started once the object or human reached the designated final position. Therefore, the latency to look at or touch the object/human could have been before the actual test start. Performing individual novel object and novel human tests separately, with the cow entering the arena each time with the object or human already in there, might enable the reliable measurement of latency. However, such modification would substantially increase the overall duration of the experiment. Generally, the approach of testing individual cows in multiple test situations in an arena is only feasible under experimental circumstances, which underlines the importance of investigating if the obtained behavioral parameters can be measured within the every-day social environment in a practical manner.

### **3.1.2. Assessment in the social environment**

The novel object test performed within the social group was a first approach to measure personality with a practical test in the home pen. Contrary to the hypothesis that from the individual test only object contact shows an association with the group test, the duration of movement in the novel arena test was also positively related to the duration of object contact in the social group. Possible reasons for this may be that in the home pen a higher level of general activity might be necessary to notice the presented novel object, and the amount of contact can also be influenced by how often a cow encounters the object. Additionally, results indicate that the timing and duration of such a group test may be an important factor to consider. The chosen test duration of 3 hours in this study was sufficient to enable object contact durations comparable to those in the individual test for each cow in the group (i.e., in the individual test max. 15 min/cow corresponds to approximately 3 hours for a group). However, cows with less competitive success might be lying longer during the day (Winckler et al., 2015) and they may not be motivated to leave a lying stall only to explore a novel object. Despite the above mentioned difficulties associated with a novel object test in the social group, the positive relationship found between individual and group test results indicates that some aspects of personality appear to be consistent between these contexts. However, the lack of temporal stability in the group test showed that the personality shown in the group can be dependent on the social background conditions.

Although the individual test results showed stability over time, the group test was not repeatable. Multiple explanations are possible for this: First, the applied group test may not reliably capture consistent individual differences. In a social context the novel object test is possibly less stressful compared to individual exposure because it does not involve social isolation. Therefore a group test might be more prone to habituation, which is partly supported by the results: in two out of three groups cows showed a decreased object contact during the second test. Second, the social group might have influenced individual behavior (Veissier et al., 1998) through processes such as social conformity, social facilitation (Webster and Ward, 2011; Stoye et al., 2012) or the effects of the social hierarchy. Results obtained in the third group indicate the influence of the social group: here more object contact was observed during the second test, which might be better understood when considering the results of the second study. Dominance calculations in

this group confirmed that in the first test two cows which had very long object contact durations were the most dominant ones. It might be speculated that once these cows contacted the object, other group members were not motivated to compete with them. These observations suggest that the interplay between dominance and personality traits, such as boldness, in specific cows might have considerable effects on the behavior of their group companions. A link between dominance, aggressiveness, and boldness (Sih et al., 2004; Holekamp and Strauss, 2016) has been found in other species, but in cattle the relationship between different personality traits has not yet been studied in detail.

In our study, only a weak relationship was found between object contact in the group and the personality trait boldness (i.e., derived from different parameters via PCA, not the single parameter object contact). Boldness is defined as the reaction to risky situations (Réale et al., 2007) and contacting a novel object is not necessarily considered risky in a group test in the home pen. The risk perceived by more shy or fearful cows may be lower if they see others contacting the object, and this could also lead to social facilitation (Webster and Ward, 2011). Furthermore, if contact with a novel object is not considered risky it might also represent the curiosity or neophobia (MacKay et al., 2014) of animals, traits slightly different from boldness. Additionally, after initial contact a novel object hanging in a group for several hours may also facilitate the occurrence of object play behavior (Held and Špinka, 2011). In conclusion, in future studies contact duration with a novel object in the group could be used as a parameter along with other measurements to characterize boldness in the home pen of cows.

### **3.1.3. Personality traits**

In line with the hypothesis, the multidimensional nature of dairy cattle personality was confirmed by the individual test results of this study. Three out of five personality traits (Réale et al., 2007) showed some correspondence to the used individual test parameters. Although PCA resulted in two components and two personality traits were extracted, based on the loadings the first trait could be interpreted in different ways: activity or exploration. According to our interpretation the second trait reflected boldness. We tested the stability of these traits after six months because existing work indicated that behavioral reactions might be more stable in adult animals than in calves. Comparing the

stability of personality traits between studies is difficult (trait names are based on interpretation), so relying on behavioral parameters may be more informative. For example, in calves most behavioral parameters reflecting the reaction to novelty were consistent between tests performed at 13 and 26 weeks of age, but not between 3 and 26 weeks of age (Van Reenen et al., 2005). Haskell et al. (2012) found in adult dairy cattle that their response to humans changes with increasing age during the productive lifetime, approximately until the middle of the first lactation. In contrast, some of the behavioral reactions of cows to social separation showed consistency over two lactations (Müller and Schrader, 2005b). For both personality traits found in the first study, only relatively few cows exhibited extreme high or low scores. A possible explanation could be that behavioral reactions to fear eliciting situations decline with age due to habituation to the farming system, and therefore the phenotypic variation reduces in the population as animals age (Haskell et al., 2014).

#### **3.1.4. Practical implications**

In this thesis, personality of adult lactating cows was considered because this might have particular relevance for the every-day management of dairy farms. The obtained personality traits showed temporal stability and a positive association with a behavioral test in the social group, underlining the relevance of personality for improving individual as well as group welfare. For example, the revealed personality traits might be considered when regrouping animals or creating new groups. It is possible that significant differences arise between groups in terms of social stress not only because of group size or stocking density (Estevez et al., 2007; Jensen and Proudfoot, 2017), but also because of the personality composition (Wolf and Krause, 2014). Therefore, future work could investigate in detail if an optimal personality composition can reduce the social stress level in a particular environment. In addition, the found traits may serve as a basis to investigate the connection between personality and individual differences in energy metabolism (Careau et al., 2008; Careau and Garland, 2012), productivity (Biro and Stamps, 2008), or methane emission (Llonch et al., 2018b) of cows. Finally, if the economic relevance and heritability of practically measurable personality traits are better known, they might be relevant for breeding (Koolhaas and Van Reenen, 2016). For the practical measurement of personality a group novel object test alone does not seem to be suitable, but it could

be an addition to long-term behavioral measurements in the home pen (e.g., using activity loggers; Ledgerwood et al., 2010; MacKay et al., 2014). Potential advantages of this test are the easy applicability and the possibility to be automatized. More specifically, closeness or contact to a novel object might be measured automatically using proximity loggers (Boyland et al., 2013), location tracking (Pastell et al., 2018) or video image analysis (Cangar et al., 2008; Guzhva et al., 2018). However, repeating such a test can lead to habituation (cf., section 3.1.2.). Also, the social environment may have an influence on the test results. Controlling for habituation by using varying objects in test repetitions and considering the results along with dominance measurements could improve the interpretability of the results. The algorithm presented in the third study might be used to obtain information on the dominance structure in the group when applying a novel object test. In conclusion, a novel object test in the social group might be useful for routinely capturing personality traits, once we better understand the influence of the social environment and other factors on the test reliability.

### 3.2. Social behavior in dairy cow groups

#### Summary at a glance: Personality in dairy cows

- ✓ Behavior of adult lactating cows in a novel arena, novel object, and novel human test showed between-individual variability and within-individual consistency over 6 months.
- ✓ Two personality traits were identified based on parameters measured in these individual tests (Activity/Exploration, and Boldness), confirming the multidimensional nature of personality in cattle.
- ✓ A practical novel object test performed within the social group of the cows showed a positive association with the duration of object contact and movement in the individual tests.
- ✓ This group test did not show temporal consistency and the repeated test indicated the impact of changing group composition on individual behavior.
- ✓ The proposed group test could be an addition to other behavioral measures for characterizing the expression of personality within the every-day social environment of cows.

### 3.2.1. Recording social interactions

This work used continuous video analysis of multiple days to provide an insight into the social interaction patterns of dairy cow groups. Moreover, to develop practical guidelines for social behavior assessment, the second study revealed the times and areas in the barn that are relevant for detecting agonistic or affiliative interactions. Although the social behavior varied over days in terms of interaction numbers as well as social networks, we found more stable patterns when aggregating 2-3 days data. This timescale supports continuous sampling over multiple days (e.g., Val-Laillet et al., 2008a, 2009) instead of short sampling periods. Based on the results of the second study, 2-3 days provide a representative snapshot of the social behavior in groups with similar size and housing conditions. Therefore this timescale was also used for the evaluation of automatic assessment of agonistic behavior in the third study.

Regarding agonistic behavior, the second and third studies showed that most agonistic interactions occur at the feed bunk, when cows are understocked at the lying stalls. However, the structure of agonistic interactions is not necessarily the same when cows compete for different resources (e.g., lying stalls, feeder, mechanical brush; Val-Laillet et al., 2008b), or when the role of aggressive behavior is to establish dominance relationships (e.g., displacement in the walking alley). Therefore, relying on the complete pen area for the assessment of agonistic behavioral patterns might introduce some bias towards the food related competition structure when the number of interactions is low in other functional areas. In addition, findings of the third study suggest that the competitiveness of cows is less variable at the feed bunk compared to other areas. Comparing social networks in different barn areas may be a useful approach for future studies to investigate how competition for different resources influences the social structure.

Contrary to agonistic interactions, grooming behavior did not show any peaks during the day and was evenly distributed between the lying stalls and the walking alley. It is often assumed that grooming represents reciprocal social bonds (i.e., friendship) in cows, but most grooming relationships were asymmetric in the second study. For the interpretation of grooming behavior it is important to consider confounding effects such as common

location preferences and spatial proximity. It is possible that cows happening to be in close proximity (e.g., lying in neighboring stalls) also perform more grooming. The connection between spatial and social preferences has not been explicitly addressed in this thesis, but other studies found a positive relationship between grooming and proximity (Gutmann et al., 2015; Boyland et al., 2016). However, this does not necessarily mean that grooming or proximity always represent social bonds. The occurrence of grooming might be influenced by the behavioral characteristics of individual cows, and also by their common spatial preferences or behavioral synchronicity. In other words, highly social cows might groom their neighbors independently from their identity, and common spatial preferences or similar activity distribution during the day might result in consistency of these neighbors. Hence, considering the individual-, dyadic-, and group-level patterns of grooming behavior in combination with agonistic interactions could facilitate the understanding of social bonds in dairy cow groups.

In this thesis, social behavior was analyzed in relatively small (11 – 20 cows) lactating cow groups, which were overstocked at the feed and water bins and understocked at the lying stalls. Therefore, it is unclear if the findings about the spatiotemporal distribution of social interactions apply in different settings, because the number and distribution of agonistic interactions can be influenced by the level of social competition. In addition, the social structure in heifer and dry cow groups may also differ from lactating cows. Finally, future work could determine if affiliative and agonistic behaviors in larger groups exhibit patterns similar to those in the small groups in this thesis.

### **3.2.2. Group- and individual-level variability**

SNA is an outstanding approach for investigating complex social relationships beyond dyadic interactions. In the second study, applying SNA as a framework for the comprehensive assessment of affiliative and agonistic behaviors provided insights both on the group- and on the individual-level. Network and node measures revealed between-group and between-individual variability, meaning that neither groups nor individuals are interchangeable in terms of social behavior. These results support the findings of the first study and provide further evidence that cows show distinct personalities and exhibit social complexity (Marino and Allen, 2017).

On the group-level a body of evidence suggests that social behavior in farm animals is more complex than just the formation of dominance hierarchies (Estevez et al., 2007; Rault, 2012; Šárová et al., 2016). The symmetric displacement and asymmetric grooming relationships observed in the second study imply that the social structure in dairy cattle groups is more complex than dominant-submissive relationships and reciprocal affiliative bonds. This complexity may partly be caused by artificial group creation and dynamic group composition changes in commercial dairies, and might show considerable differences to stable cattle groups in nature (Bouissou, 1980; Lazo, 1994). Previous work under modern management settings suggested that frequent neighbor cows at the feeder displace and groom each other more than non-neighboring cows (Val-Laillet et al., 2009). This would mean that networks based on neighbor information, displacement, and grooming are similar. We did not consider neighbor networks in the second study, and we did not find any correlation between agonistic and affiliative networks. Our study implies that different processes regulate agonistic and affiliative interactions and to describe the complete social experience of cows it is not sufficient to rely only on one of these behaviors. Therefore, this thesis focused on combining measures from affiliative and agonistic networks into the newly developed balance index. The aim of the balance index is to describe the perspective of the individual cow as part of the social group: First, what does it experience in terms of social behavior, how do the group members react to it? To describe this, a ratio of received affiliative and agonistic interactions was applied. Second, in the same manner, the reactions of the cow to the others in the group were described with the ratio of given affiliative and agonistic interactions. The balance index provides a different perspective than the classical concept of dominance or friendship. In this thesis, balance index is proposed as a first approach to describe the complete social experience of cows, and the stability between observation periods indicates that it might further our understanding of how the welfare of cows is related to social behavioral processes in the group. However, to better understand the practical relevance of the balance index, further validation with physiological measures (e.g., heart rate variability, Kovács et al., 2014; or long-term cortisol measures, Heimbürge et al., 2019) is needed.

The individual-level variability in the social behavior of cows is recognized as an important area of research (Marino and Allen, 2017). Recent work showed that the contact structure

of dairy cattle groups is heterogeneous, which can also have practical relevance for disease transmission (de Freslon et al., 2019). In the second study, node level measures showed considerable differences between cows in affiliative as well as agonistic networks, and networks of subgroups expressed temporal stability. Therefore, in future studies SNA might be a practicable tool to provide measures for personality traits such as sociability or aggressiveness (Réale et al., 2007). For example, the OUT-Degree (number of partners) and OUT-Strength (number of interactions) measures may be used as a proxy for aggressiveness, as a high number of initiated agonistic interactions with multiple partners could reliably capture aggressive behavioral tendencies. The corresponding measures in affiliative networks may be used to represent the sociability of cows. In addition, the IN-Degree measures of affiliative behavior could be used to test the existence of concepts such as popularity. Finally, the IN measures of agonistic behavior may be used to identify if specific cows are bullied in the group and if this is related to their stress levels. When we observed the subgroups of cows which were present at both study periods 6 months apart, we found stability in the individual social behavioral characteristics. The temporal stability of dominance, grooming index, and the developed balance index (despite the changes in the complete social environment) indicates that these behaviors show personality trait characteristics. Dominance has already been discussed as a potential sixth personality trait (Finkemeier et al., 2018) and our results also point into this direction. The automated detection of dominance hierarchy in groups using the algorithm presented in the third study may enable more detailed investigations concerning the stability of dominance in different groups over longer periods of time.

One aspect not considered in detail in this thesis is the relationship between personality traits and social behavior. As mentioned above, social network measures of individual cows may be used to derive personality traits such as aggressiveness or sociability. But in addition, it would be interesting to investigate the relationship between the social network position and boldness, activity, or exploration scores of individual cows (Krause et al., 2010; Wolf and Krause, 2014). Future research could also study if the social network structure of a group is associated with the number of group members with high activity or boldness scores.

### **3.2.3. Practical implications**

It has been suggested that changes in the frequencies of social interactions may provide cues about welfare problems (Mench and Mason, 1997). Accordingly, SNA could be used as a tool to develop group- and individual-level welfare markers based on the routine measurement of social behavior and its changes (Koene and Ipema, 2014). On the group-level, investigating how the network structure of cows is influenced by group composition changes using dynamic social network analysis (Pinter-Wollman et al., 2014; Farine, 2018) could be particularly relevant. However, such analysis approaches and the practical application of social behavior as a welfare marker require long-term data collection, feasible using automatic data collection methods. Multiple studies which made use of SNA in dairy cattle relied on automated data to infer social relationships: Dyadic distances based on proximity logger data (Boyland et al., 2016) were used as a proxy for affiliative interactions, whereas different tracking systems were applied to collect data on closeness or avoidance relationships within a group (Gygax et al., 2010; Koene and Ipema, 2014; Chen et al., 2015). However, these methods are often only used for the construction of undirected networks. The results of the second study have shown that the direction of social interactions is an important factor to consider. Accordingly, the replacement detection algorithm presented in the third study could be useful for routinely creating and analyzing directed agonistic networks in the future, or to study neighbor relationships at the feed bunk. Moreover, high resolution location tracking (Pastell et al., 2018) or video image analysis (Guzhva et al., 2018) might enable automated data collection for affiliative as well as agonistic networks, and the joint analysis of these networks (Smith-Aguilar et al., 2018) may also provide new insights. On the individual-level, consistent variation in social behavioral characteristics may influence how specific cows adapt to farming conditions. For example, cows which generally have strong affiliative bonds could be more sensitive to group composition changes, compared to those with weak associations. Furthermore, dominant cows may differ in their level of aggressiveness thereby potentially influencing the level of agonistic interactions in a particular group. Therefore, characterizing social personality types using SNA, and taking this information into account when creating and managing groups could help to reduce social stress in future dairy cattle husbandry systems.

**Summary at a glance: Social behavior in dairy cow groups**

- ✓ Continuous video observation of social interactions over 2-3 days provided a reliable snapshot about the affiliative and agonistic behavior in free-stall housed dairy cow groups.
- ✓ Most agonistic interactions occurred at the feeder, highlighting the relevance of this area for automatic social interaction detection.
- ✓ Social network analysis revealed between-group and between-individual differences in affiliative as well as agonistic behavior that were stable over 6 months. This provides evidence for social complexity beyond dyadic interactions and indicates distinct personality traits related to social behavior.
- ✓ Affiliative and agonistic networks did not show any association, and many symmetric displacement and asymmetric grooming relationships were observed.
- ✓ Network measures were used in a newly developed balance index to combine affiliative and agonistic interactions into one measure of complete social experience.

### **3.3. Automatic recording of agonistic interactions**

#### **3.3.1. Challenges and opportunities**

The previous sections provided evidence that recording social interactions of cows can reveal important individual- and group-level behavioral processes. Specifically, these interactions may be used to characterize between-individual variability in traits such as aggressiveness or sociability, and also to investigate the impact of group-level social structure on animal welfare. However, according to the second study, continuous data collection in the entire pen and aggregating social interactions across 2-3 days is recommended to gain a reliable snapshot. This is extremely time- and labor intensive using human video observations. Therefore, the third study set out to facilitate the transfer of the theoretical knowledge gained in the first and second study into applied settings.

The third study investigated the practical applicability of an electronic feeder system to automatically measure agonistic behavior at the individual- and group-level. This feeder system has previously been used in several studies for general feeding behavior measurements (Schirmann et al., 2011; Crossley et al., 2017; Neave et al., 2017). An alternative usage of the electronic bins is to monitor social competition at the feed bunk, using a specific replacement detection algorithm (Huzzey et al., 2014). In order to put the practical relevance of this algorithm into perspective, this thesis assessed the proportion of replacements from all agonistic interactions in multiple cow groups and facilities. Replacements were proven to be a particularly useful social interaction type to start the development of automatic long-term detection methods, because they consisted the majority of agonistic interactions at the feed bunk, and in most groups the majority of all agonistic interactions in the pen. Therefore, the wide use of this algorithm could facilitate investigations regarding how agonistic interactions within a group relate to specific characteristics of cows such as health or productivity.

Automatic data collection methods are becoming increasingly popular in agriculture, as precision livestock farming is expanding (Berckmans, 2014). Electronic systems have the advantage of providing data seemingly effortlessly. However, it is often neglected that a thorough validation, proper system maintenance, and regular checks for data coherence

and plausibility are necessary, because relying on automatically collected data increases the risk of systematic bias caused by technical errors or suboptimal parameter settings. Therefore, this thesis improved the existing replacement detection algorithm by providing detailed data preparation guidelines and validated the optimal parameter settings in groups with varying stocking densities in two different facilities. The presented workflow provides suggestions for controlling technical errors and a detailed description of data preparation that can be used in any barn with the same system. In addition, the validation process might be used as a guideline when evaluating different systems for similar purposes.

This thesis provided proof that the replacement detection algorithm is a useful tool for the measurement of individual-level agonistic behavior and group-level dominance structure of cows. Besides the high recall and precision in detecting replacements at the feed bunk, the algorithm provided a very good approximation of the dominance scores based on all agonistic interactions. However, replacement based dominance scores showed less variability between individuals, indicating that the difference between the competitiveness of cows is smaller for replacements compared to other agonistic interactions in the pen. The algorithm performed well under the housing conditions in the two facilities in the study, but it has not yet been tested under considerably different settings. For example, the relevance of replacements at the feed bunk might change if cows are overstocked for lying stalls, or if the stocking density increases at the bins. Furthermore, we currently have no knowledge if the same parameter settings are applicable in larger cow groups. Given that the used electronic feeder system is mostly installed in research facilities, the group size used for the validation in this thesis is probably relevant for most users of the system.

### **3.3.2. Practical implications**

The presented method opens new possibilities for studying the relevance of agonistic behavioral patterns and dominance for the health and welfare of dairy cattle. Existing work indicates several topics that could be further investigated in detail using the automated dominance detection with the algorithm. For example, dominance might be related to elevated or decreased level of glucocorticoids according to the concept of

allostasis (Goymann and Wingfield, 2004; Rubenstein and Shen, 2009). Changes in social status might be associated with reproductive performance (Dobson and Smith, 2000), and an unpredictable competitive social environment was associated with higher uterine disease risk after calving in multiparous cows (Proudfoot et al., 2018). Empirical studies in pigs also found that psychosocial stress can have an effect on the immune function and inflammatory responses (Gimsa et al., 2018). Furthermore, a study found that low-ranking cows had significantly lower survival rates to lameness than high ranking ones (Galindo et al., 2000). Finally, according to a recent review, feeding behavior and therefore productivity and welfare of cows might be modulated by dominance and aggressiveness traits (Llonch et al., 2018a). However, for such investigations it is important to bear in mind that for each individual the costs and benefits of the social status may depend on the group composition. Therefore, interpreting social status within the context of the given group is beneficial to better understand the connection between social behavior, health and welfare. Automatically measuring replacements with electronic bins enables considering the dominance status of cows within the context of a given group. The continuous detection of replacements at the feed bunk in multiple groups in a facility would also allow for investigating the dynamics of dominance status (e.g., using the Elo-rating, Sánchez-Tójar et al., 2018) and agonistic networks of cows over longer periods, in reaction to various impact factors. In addition, using the replacement detection algorithm to create and analyze agonistic social networks may help to better understand the association between aggressiveness and dominance as different personality traits (Holekamp and Strauss, 2016). Such information would facilitate the integration of social behavior into welfare measures and thus may allow for an optimized group management strategy on commercial dairy farms.

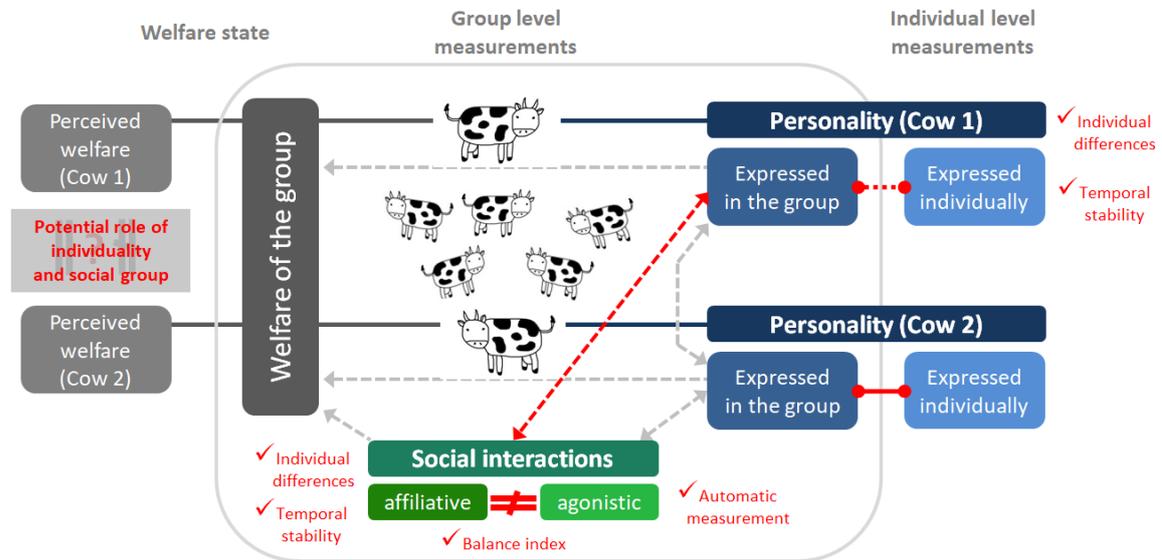
**Summary at a glance: Automatic recording of agonistic interactions**

- ✓ An algorithm using occupation data from electronic feed and water bins was validated in multiple facilities for recording agonistic interactions on the individual-level and detecting the dominance hierarchy on the group-level.
- ✓ Independently from facility and stocking density, a 20 to 30 s time interval between the occupancy of two cows at one bin can be used to reliably estimate the number of competitive replacements and the dominance structure of groups.
- ✓ This algorithm is a practical tool to be used in further studies to investigate the relationship between aggressiveness, dominance, and welfare of dairy cows.

### 3.4. Conclusions

This thesis set out to combine individual- and group-level analysis of behavior to provide insights on how personality and social behavior of dairy cows is related to their welfare.

The main conclusions of the work are summarized below and illustrated in **Figure 3**.



**Figure 3.** Possible relationships between welfare, social behavior, and personality on individual- and group-level, based on an example of two cows in a group. The analyzed relationships and main results are shown in red. Dashed lines indicate partial relationships.

The first two studies provided evidence that individual cows, and also dairy cow groups show specific behavioral variation. Individual behavioral tests revealed two distinct personality traits, stable over time, and the consistent individual differences in social behavior further supported the multidimensional nature of cattle personality. Some aspects of personality were also identified within the social group using a practical test. However, the repetition of the group test indicated the impact of changing group structure on the expression of individual personality. To better understand the complex social environment of cows, social network analysis and behavioral indices (i.e., dominance, grooming index) were used to gain detailed insights into affiliative and agonistic behaviors on individual-, dyadic- as well as group-level. On the individual-level, consistent differences were found not only in the agonistic, but also in the grooming behavior of cows, supporting the existence of related personality traits. On the dyadic-level, the obtained symmetric displacement and asymmetric grooming relationships threw doubt on the classical interpretation of dominance and affiliative bonds in dairy

cow groups. The group-level structure of affiliative and agonistic networks did not show any association, indicating that one social interaction type does not capture the complete social environment. The newly developed balance index combines affiliative and agonistic network measures into one complete descriptor of sociality. This index could be used in future work to measure how the complete social experience of cows within a group is related to their welfare.

Besides advancing the theoretical knowledge about individual- and group-level behavioral variation in dairy cows, this thesis also aimed to develop methods to facilitate the practical application of the results. The group test proposed in the first study could be an easy-to-use addition to other personality measurements in the home pen, especially if the social structure of the group is known. The second study provided guidelines for the assessment of agonistic and affiliative behavior in free-stall barns based on continuous video analysis of all social interactions in multiple groups. In addition, the third study validated an electronic feeder system in different facilities and groups, thereby showing that it can reliably detect individual- and group-level agonistic behavioral patterns. The presented data collection, preparation and analysis guidelines enable the wider application of social behavior in future investigations regarding individual- and group-level welfare.

The results of this thesis indicate that individual cows consistently differ in their behavioral reactions in various ways, corresponding to the emergence of a complex social environment in the barn. This also highlights the importance of considering both the level of individuals and the level of social groups for the assessment of dairy cattle welfare, in contrast to focusing solely on farm-level evaluation. Further development of different methods for the automatic long-term measurement of individual and group-level behavior can make such assessments suitable for practical application in the future.

## Summary

In modern dairy husbandry systems cows are grouped and regularly regrouped according to their milk production and nutritional needs. Although it is recognized that individuals within a group may significantly differ regarding their needs and welfare states, currently little consideration is given to individuality and social behavior when managing groups of dairy cows. On the individual-level, cows exhibit consistent behavioral differences (i.e., personality), but the manifestation and role of these differences within the social group is not well understood. Moreover, knowledge about complex social interactions beyond dyadic encounters in groups of dairy cows is still limited. However, with increasing availability of advanced technical solutions in livestock farming, automatic individual- and group-level data collection can facilitate management practices that better serve the welfare of cows. Therefore, this thesis investigated the behavioral characteristics of lactating dairy cows using individual- as well as group-level analysis, to extend our knowledge about personality and social behavior and to suggest practical improvements for modern dairy systems.

The **first chapter** briefly introduces the connections between individual- and group-level welfare. This is followed by an introduction to the concept of animal personality and the current knowledge regarding its measurement methods and practical relevance in dairy cattle is outlined. An overview is given about agonistic and affiliative behavior in dairy cows as well as an explanation of social network analysis. Finally, traditional and new methods for recording social behavior in dairy cattle groups are presented.

The **second chapter** presents three experimental studies considering personality, social behavior, and the automatic detection of agonistic behavior in dairy cow groups:

In the **first study** the temporal and contextual stability of behavior was tested in individual and group situations. The existence of consistent individual-level behavioral variation between cows was supported by the findings and multiple personality traits were confirmed. These were stable over six months and aligned with the five traits in the framework of animal personality. Behavior between the individual and group test contexts showed consistency. However, the group test has not been repeatable, highlighting the effect of habituation and the influence of the social group on individual

responses to novelty. In conclusion, specific aspects of personality might be measured with a novel object test in the social group, but it is beneficial to interpret the results in relation to the social position of the individual in the group.

Complementing the personality measurements in the first study, the **second study** revealed consistent individual variability in the social behavior of cows. In addition, social network analysis showed that affiliative and agonistic behaviors follow different patterns on the group-level. Therefore, a new balance index was developed to combine these two behaviors into one measure and to consider the relationship between received and provided social interactions for each cow within a group. Moreover, this study provided guidelines for future investigations of the social environment in groups of dairy cattle by suggesting that 2-3 days of continuous observation is sufficient to gain a reliable picture on social behavioral patterns.

To pave the way for the practical measurement of personality and social behavior, the **third study** considered the analysis of agonistic behavior and dominance hierarchy in dairy cattle groups based on automatic measurements. Focusing on agonistic interactions in the feed bunk area, this work validated an algorithm that uses combined electronic feed and water bin data in multiple facilities. With the described parameter settings and filtering methods, the algorithm can widely be used in different facilities to detect agonistic interactions in groups with varying stocking densities. In addition, this study provided evidence that the algorithm gives a reasonable estimate of the group dominance hierarchy, thereby enabling further investigations regarding the relationship between social behavior, personality, and welfare.

The **third chapter** comprises the general discussion of the experimental studies and their interconnections. This chapter also presents the practical implications of the results and outlines how they might be used in future research on personality and social behavior. For each study the main results are summarized in a highlighted text box.

In conclusion, this thesis emphasizes the behavioral differences between cows on the individual- and group-level and presents novel methods that will help to transfer this new knowledge into practical application. The insights gained through this thesis will help to improve the welfare of dairy cattle by minimizing the costs and maximizing the benefits of living in a group.

## Zusammenfassung

In modernen Milchviehhaltungssystemen werden Kühe nach ihrer Milchproduktion und ihren Ernährungsbedürfnissen zusammengestellt und regelmäßig umgruppiert. Obwohl bereits bekannt ist, dass sich Einzeltiere innerhalb einer Gruppe in Bezug auf ihre Bedürfnisse und ihren Wohlbefinden erheblich unterscheiden können, wird derzeit bei dem Management von Milchkuhgruppen nur wenig auf Individualität und Sozialverhalten geachtet. Kühe zeigen konsistente individuelle Verhaltensunterschiede (d.h. Persönlichkeit), aber die Manifestation und Rolle dieser Unterschiede innerhalb der sozialen Gruppe ist nicht ausreichend untersucht. Darüber hinaus ist das Wissen über komplexe soziale Interaktionen jenseits von dyadischen Interaktionen in Milchviehgruppen immer noch begrenzt. Die zunehmende Verfügbarkeit innovativer technischer Lösungen in der Tierhaltung ermöglicht die automatische Datenerfassung auf Einzeltier- und Gruppenniveau und kann Managementpraktiken erleichtern, die dem Wohlbefinden von Kühen besser dienen. In dieser Arbeit wurden daher Verhaltensmerkmale laktierender Milchkühe anhand von Analysen auf Einzeltier- und Gruppenebene untersucht, um unser Wissen über Persönlichkeit und Sozialverhalten zu erweitern und praktische Empfehlungen für moderne Milchviehsysteme bereitzustellen.

Das **erste Kapitel** stellt die Zusammenhänge zwischen dem Tierwohl auf Einzeltier- und Gruppenebene vor. Daran schließt sich eine allgemeine Einführung in das Konzept der Persönlichkeit bei Tieren an, zusammen mit den Messmethoden und der praktischen Relevanz beim Milchvieh. Es wird ein Überblick über sozio-negatives und sozio-positives Verhalten bei Milchkühen, sowie eine Einführung in die Methode der „Sozialen Netzwerkanalyse“ gegeben. Abschließend werden traditionelle und innovative automatisierte Methoden zur Erfassung des Sozialverhaltens in Milchviehgruppen vorgestellt.

Das **zweite Kapitel** stellt drei experimentelle Studien vor, die die Persönlichkeit, das Sozialverhalten und die automatische Erkennung von sozio-negativem Verhalten in Milchkuhgruppen untersuchen:

In der **ersten Studie** wurde die zeitliche und kontextuelle Stabilität des Verhaltens in Einzel- und Gruppensituationen getestet. Das Vorhandensein einer konsistenten

Verhaltensvariation zwischen den Kühen auf individueller Ebene wurde durch die Ergebnisse gestützt, und mehrere Persönlichkeitsmerkmale wurden bestätigt. Diese waren über sechs Monate hinweg stabil und stimmten mit bereits definierten Merkmalen von Persönlichkeit bei Tieren überein. Das Verhalten in Einzel- und Gruppentest war konsistent. Die Aussagekraft des wiederholten Gruppentests hinsichtlich der zeitlichen Konsistenz des Verhaltens war jedoch begrenzt, da Habituation und eine teilweise veränderte Gruppenzusammensetzung die individuellen Reaktionen möglicherweise stark beeinflusst haben. Zusammenfassend können bestimmte Aspekte der Persönlichkeit mit dem Novel Objekt Test in der sozialen Gruppe gemessen werden. Es ist jedoch vorteilhaft, die Ergebnisse zusammen mit Informationen über die Dominanzstruktur zu interpretieren.

Die **zweite Studie** ergänzte die Persönlichkeitsmessungen in Studie 1 und zeigte eine konsistente individuelle Variabilität im Sozialverhalten von Kühen. Darüber hinaus zeigte die Soziale Netzwerkanalyse, dass das sozio-positive und das sozio-negative Verhalten auf Gruppenebene unterschiedlichen Mustern folgen. Daher wurde ein neuer sozialer Balance-Index entwickelt, der beide Formen sozialer Interaktion berücksichtigt und die Beziehung zwischen den erhaltenen und ausgeführten sozialen Interaktionen für jede Kuh innerhalb einer Gruppe darstellt. Darüber hinaus lieferte diese Studie Empfehlungen für kommende Untersuchungen des Sozialverhaltens, indem nachgewiesen wird, dass eine 2- bis 3-tägige kontinuierliche Beobachtung nötig ist, um ein verlässliches Bild über die sozialen Verhaltensmuster in Milchviehgruppen zu erhalten.

Um den Weg für eine einfache Feststellung von Persönlichkeit und Sozialverhalten unter Praxisbedingungen zu ebnen, untersuchte die **dritte Studie** das sozio-negative Verhalten und die Dominanzhierarchie in Milchviehgruppen auf der Grundlage automatisch erfasster Daten. Diese Arbeit konzentrierte sich auf sozio-negative Interaktionen im Futterbereich und validierte in mehreren Milchviehbetrieben einen Algorithmus, der den Aufenthalt und die Verdrängung an elektronische Futter- und Wassertrögen analysiert. Mit den beschriebenen Parametereinstellungen und Filtermethoden kann der Algorithmus in Betrieben mit entsprechender technischer Ausrüstung eingesetzt werden, um das sozio-negative Verhalten in Gruppen mit unterschiedlichen Besatzdichten zu erfassen. Darüber hinaus lieferte diese Studie den Nachweis, dass der Algorithmus eine

korrekte Einschätzung der individuellen Rangposition der Tiere einer Herde ermöglicht, wodurch weitere Untersuchungen hinsichtlich des Zusammenhangs zwischen sozialem Verhalten, Persönlichkeit und Tierwohl ermöglicht werden.

Das **dritte Kapitel** beinhaltet die übergreifende Diskussion der experimentellen Studien und ihrer Zusammenhänge. In diesem Kapitel wird auch die praktische Relevanz der Ergebnisse dargestellt und beschrieben, wie sie in zukünftigen Arbeiten zu Persönlichkeit und Sozialverhalten eingesetzt werden können. Für jede Studie werden die Hauptergebnisse in einer hervorgehobenen Textbox präsentiert.

Zusammenfassend betont diese Arbeit die Verhaltensunterschiede zwischen Kühen auf Einzeltier- und Gruppenebene und stellt neue Methoden vor, die helfen werden, dieses neue Wissen in der Praxis umzusetzen. Die Erkenntnisse aus dieser Arbeit werden dazu beitragen, das Wohlbefinden von Milchvieh zu verbessern, indem die Nachteile des Zusammenlebens in einer Gruppe minimiert und die Vorteile maximiert werden.

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## Theses

### Objectives of research

In modern dairy husbandry systems social groups are created and regularly changed according to the milk production and nutritional needs of cows. It is now recognized that there are consistent differences between cows within a group and that both individuals and groups may significantly differ regarding their welfare states. Nonetheless, currently little consideration is given to individuality and social behavior when managing groups of dairy cows. The personalities of individual cows and the group-level patterns of affiliative and agonistic interactions are potentially interconnected and may also influence welfare. However, little knowledge exists about the personality traits dairy cows express within the social environment, and it is unclear if these traits show temporal stability. Moreover, complex social interactions in groups of cattle beyond dyadic encounters are understudied, partly due to the time consuming nature of data collection. In contrast to agonistic relationships, knowledge about the role of affiliative bonds is limited, and no measure exists to integrate positive and negative interactions into one complete descriptor of sociality. Besides the understanding of behavioral processes in dairy cow groups, standardized and practical methods are needed to enable the application of this knowledge. With the increasing availability of advanced technical solutions in livestock farming, automatic data collection can facilitate management practices that better fit the needs of cows.

Therefore, in this thesis a common framework for individual- and group-level analysis of social behavior was used to better understand how the behavioral characteristics of individual cows and the behavior in the social group may influence welfare. In addition, the thesis focused on developing novel methods, facilitating the practical assessment of personality and social behavior in dairy cattle. Accordingly, three main objectives were assessed:

- 1) Investigating personality in adult lactating cows. Specifically, assessing if (a) personality traits, revealed by a number of classical individual tests, express stability over a longer period of time, and (b) personality shows consistency between individual and group contexts, measured by a developed practicable group test in the home pen.

- 2) The comprehensive analysis of the social environment in a free-stall barn. This included (a) determining a suitable time scale for the analysis of affiliative and agonistic interactions, (b) investigating the relationship between the structure of these two behaviors and also combining them into one measure, and (c) assessing the long-term temporal stability of individual social behavioral characteristics.
- 3) Facilitating the automated assessment of agonistic behavior on the group-level by validating an electronic-feeder-based algorithm for detecting the dominance hierarchy in lactating dairy cow groups in different facilities.

### **Main findings**

Regarding the temporal and contextual stability of personality the following main findings were published in PLOS ONE 2018; 13(10):e0204619.

- Behavior of cows in a novel arena, novel object, and novel human test showed between-individual variability and within-individual consistency over 6 months.
- Two personality traits (Activity/Exploration and Boldness) were identified based on parameters measured in the individual tests, confirming the multidimensional nature of personality in cattle.
- A practical novel object test performed within the social group of the cows showed a positive association with object contact and movement in the individual tests.
- This group test did not show temporal consistency and the repeated test indicated the impact of changing group composition on individual behavior.
- The group test could be an addition to other behavioral measures to characterize the expression of personality in the every-day social environment of cows.

The analysis of the social environment in dairy cow groups resulted in the following main findings, which were published in Applied Animal Behaviour Science 2019; 210:60-67.

- Continuous video observation of social interactions over 2-3 days provided a reliable snapshot about the affiliative and agonistic behavior in free-stall housed small dairy cow groups.
- Most agonistic interactions occurred at the feeder, highlighting the relevance of this area for automatic social interaction detection.

- Social network analysis revealed between-group and between-individual differences in affiliative as well as agonistic behavior that were stable over 6 months. This provides evidence for social complexity beyond dyadic interactions and indicates distinct personality traits related to social behavior.
- Affiliative and agonistic networks did not show any association, and on the dyadic level many symmetric displacement and asymmetric grooming relationships were observed.
- Network measures were used in a newly developed balance index to combine affiliative and agonistic interactions into one measure of social experience.

Finally, the following key findings are included in the third study, which was submitted to *Journal of Dairy Science* (under review):

- An algorithm using data from electronic feed and water bins was validated in multiple facilities for recording agonistic interactions on the individual-level and detecting the dominance hierarchy on the group-level.
- A 20 to 30 s time interval between the occupancy of two cows at one bin can be used to reliably estimate the number of competitive replacements and the dominance structure of groups.
- This algorithm is a practical tool to be used in further studies to investigate the relationship between aggressiveness, dominance, and welfare of dairy cows.

## **Conclusions**

This thesis demonstrates that dairy cows consistently differ in their behavioral reactions in various ways, corresponding to individual personality and the emergence of a complex social environment in the barn. The findings suggest that the level of individuals and the level of social groups should also be taken into account when assessing dairy cattle welfare, in contrast to focusing solely on farm-level evaluation. The automatic detection of agonistic behavior and dominance is one important step in the direction of practically recording individual and group-level variation in social behavior. The knowledge and new methods presented in this thesis will help to improve the welfare of dairy cattle by minimizing the costs and maximizing the benefits of living in a group.

## Curriculum Vitae

### Borbala Foris

**Date of birth:** 04.11.1987  
**Place of birth:** Budapest, Hungary  
**Nationality:** Hungarian

### Education

2015 – 2019 **PhD Studies**  
 Institute of Genetics and Biometry, FBN, Dummerstorf, Germany  
 Faculty of Agricultural and Environmental Sciences, University of Rostock

2012 **Doctor of Veterinary Medicine**  
 University of Veterinary Medicine, Budapest, Hungary  
 Thesis: Evaluating Welfare Quality in Hungarian Dairy Herds

2006 – 2012 **Studies of veterinary medicine**  
 University of Veterinary Medicine, Budapest, Hungary

### Research Experience

2015 – 2019 **Graduate research**, Institute of Genetics and Biometry, FBN, Dummerstorf, Germany

- Performed and analyzed behavior tests for personality measurement in dairy cattle
- Gained experience in the calibration and evaluation of a UWB tracking system in a barn environment
- Analyzed continuous video footage to record social interactions between cows with Mangold Interact®

2017 **Research visit**, Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver BC, Canada.

- Investigated the connection between illness and social behavior in dairy cattle

2010 – 2012 **Undergraduate research**, Department of Animal Hygiene, Herd health and Veterinary Ethology, University of Veterinary Medicine, Budapest

- Evaluated the welfare status of several Hungarian dairy farms using the Welfare Quality Protocol.
- Gained experience in the assessment of body condition score and lameness of dairy cattle.
- Organized a field-trip to Germany to compare the usability of the Welfare Quality Protocol regarding farm size.

- 2010      **Internship**, Clinic for Cattle, University of Veterinary Medicine, Hannover, Germany
- Participated in diagnostic work and surgical treatment of cattle

### **Awards**

- 2016      FBN Day of the Doctoral Students Best Presentation Award
- 2012      Student's Research Congress First Prize (Dr. Kovács Ferenc Award)

### **Additional Training**

- 2018      Statistics Course (SAS, R), Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany
- 2016      Workshop: Social Network Analysis, Edward Grey Institute, University of Oxford, Oxford, United Kingdom
- 2016      Workshop: Scientific Writing, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany
- 2015      Introduction to Biometry 3 (SAS), Thünen Institute, Braunschweig, Germany
- 2014      MOOC (Massive Open Online Course):
- Agriculture And The World We Live In, Massey University, New Zealand
  - Animal Behaviour and Welfare, University of Edinburgh
- 2013      Internship: Tierarztpraxis am Ith, Copenbrügge, Germany
- 2012 – 2013      Internship: Small Animal Veterinary Praxis Kirsten Türk, Duingen, Germany

### **Languages**

- English: high level
- German: high level
- French: intermediate level
- Hungarian: native speaker

### **Technical skills**

- Video analysis (Mangold Interact)
- Social network analysis, statistical analysis (R)
- UWB Tracking data collection (Ubisense, Noldus – TrackLab)

## List of publications

### Peer-reviewed journal articles:

Foris, B.; Zebunke, M.; Langbein, J.; Melzer, N. (2018): *Evaluating the temporal and situational consistency of personality traits in adult dairy cattle*. PLOS ONE. 13(10):e0204619

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### Peer-reviewed conference abstracts:

Foris, B.; Langbein, J.; Melzer, N. (2018): *Patterns of affiliative and agonistic interactions in dairy cattle*. In: Book of abstracts of the 69th Annual Meeting of the European Federation of Animal Science (EAAP): Dubrovnik, Croatia, August 27-31, 2018. Wageningen Academic Publishers, Wageningen. (ISBN: 978-90-8686-323-5):415

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Foris, B.; Trißl, S.; Graunke, K. L.; Langbein, J. (2015): *Differences in observable traits during veterinary treatment in dairy and beef cattle*. In: Proceedings: XXV Jubilee International Congress of the Hungarian Association for Buiatrics, September 13-16, 2015, Budapest (Magyar Allatorvosok Lapja; 137.2015, Suppl.1) (ISBN: 978-963-12-3377-3) (137): 100-102

**Non-peer-reviewed conference abstracts:**

Foris, B.; Zebunke, M.; Langbein, J.; Melzer, N. (2016): *Using Social Network Analysis to study affiliative and agonistic relationships in dairy cattle*. In: Aktuelle Arbeiten zur artgemäßen Tierhaltung 2016 KTBL, Darmstadt. (ISBN: 978-3-945088-25-8) (511):264-266

Foris, B.; Trißl, S.; Zebunke, M.; Langbein, J.; Puppe, B. (2016): *The effect of personality and social structure on the welfare of dairy cattle*. Animal Social Network Analysis Workshop, Edward Grey Institute of Field Ornithology (EGI), 6.1.2016-8.1.2016, Oxford, United Kingdom

Foris, B.; Zebunke, M.; Langbein, J.; Melzer, N. (2016): *Individual and within-group personality measurements in dairy cattle*. 17th Day of the Doctoral Student, Leibniz Institute for Farm Animal Biology (FBN), Schriftenreihe / Leibniz-Institut für Nutztierbiologie 25: 5-8

**Other contributions:**

Foris, B. (2017): *Studying personality and social structure in dairy cattle*. Presentation during research visit at the University of British Columbia, Faculty of Land and Food Systems, Animal Welfare Program, 18.7.2017, Vancouver, Canada

Foris, B. (2015): *Personality and social welfare in dairy cattle*. Presentation, 16th Day of the Doctoral Student, Leibniz Institute for Farm Animal Biology (FBN), 4.11.2015-4.11.2015, Dummerstorf, Germany