EFFECT OF REGROUPING ON SOCIAL BEHAVIOUR AND MILK PRODUCTION OF MID-LACTATION DAIRY COWS, AND INDIVIDUAL VARIATION IN AGGRESSION

by

Kalab N. Tesfa

B. Sc., The University of Asmara, 1999

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Animal Science)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

March 2013

Abstract

Dairy cows are often mixed into new social groups for management reasons, but this is recognized as a cause of social stress. The objectives of this study were to investigate the effect of regrouping on social behaviour, self-licking and milk production of mid-lactation cows introduced in pairs, and to see whether individual variation in aggression is consistent before and after regrouping. In 7 replications (n=7), 14 mid-lactation cows were introduced in pairs into an established group of filler (resident) cows. After regrouping, agonistic contacts and displacement of the introduced cows increased during the first 3 and 2 days, respectively. Compared to baseline (the day before regrouping), the number of social licking events between the introduced cows and the resident cows in the pen did not change after regrouping, but the proportion of social licking between the two introduced cows increased sharply after regrouping (38 ± 9 %) compared to baseline (10 ± 9 %). Duration of social licking decreased declined after regrouping; whereas, self-licking increased on the day of regrouping. Compared to the resident cows, milk production of the introduced cows significantly decreased on the first 2 days after regrouping, and showed a negative linear association with agonistic contacts received and with displacements lost. Two measures of aggressive behaviour (proportion of agonistic contact initiated and proportion of displacements won) were relatively consistent before and after regrouping (R^2 = 0.75 and 0.68 respectively), suggesting that the differences reflected individual differences in aggressiveness, rather than social status within a given group. In the present experiment, low-and high-aggressive individuals were not different in milk production, social licking and age at first calving, but low-aggressive cows had higher 305-day projected milk production (12,928.0 ± 580 kg) than high-aggressive cows (10,530.0 ± 530 kg). Individual variation in aggression was not associated with body weight, although the heaviest cow in the group won all encounters before and after regrouping. The findings of this study

provide the first insights that introducing cows in pairs may mitigate the effects of social stress during mixing.

Preface

The effects of regrouping on social behaviour, self-licking and milk production of cows introduced in pairs into a new social group. The study was designed collaboratively by Kalab Tesfa, Dr. Dan Weary and Dr. Marina von Keyserlingk. Kalab Tesfa executed the experiment and collected all data. Kalab Tesfa was primarily responsible for data analysis, for interpretation results and preparation of the thesis under the supervision of Drs. David Fraser, Marina von Keyserlingk and Doug Veira. Co-authors edited drafts of the thesis. Kalab Tesfa successfully completed the ethics training requirements of the Canadian Council on Animal Care (CCAC) / National Institutional Animal User Training (NIAUT) Program (Certificate number: 4545-10; Date Issued: December 15, 2010.

This research was conducted under the animal care protocol A10-0162 for Dairy Cattle Welfare: Behaviour, lameness and disease, issued by the University of British Columbia Committee on Animal Care.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOVA = Analysis of Variance

CDIC = Canadian Dairy Information Center

GLM = Generalized Linear Model

HPA-axis = Hypothalamic-Pituitary Adrenal axis

DI = Displacement Index

IAI = Initiated Aggression Index

LAL = Long Attack Latency

ME = Mature Equivalent

CCAC = Canadian Council on Animal Care

LSMEAN = Least Square Mean

OIE = World Organisation for Animal Health

R^2 = Coefficient of determination

SAL = Short Attack Latency

SAS = Statistical Analysis System

TMR = Total Mixed Ration

Acknowledgements

I would like to extend my thanks to the Animal Welfare Program at University of British Columbia for giving me a wonderful opportunity to experience a graduate student life in a world-class university.

I would like to thank my principal supervisor David Fraser for his caring and professional guidance, his constructive comments and criticism on my thesis, and encouraging me to peruse my studies. David, your class also gave me the first impression in animal welfare. A lot of thanks also go to my co-supervisor, Nina von Keyserlingk, for her passionate and educational advice, encouragement and comments and for her extraordinary contribution in designing the experimental study and the constructive comments on the thesis. Many thanks go to Douglas Veira, a supervisory committee member whose daily visits at the dairy centre during my data collection, especially at pen 21, was really fun and comforting. To Dan Weary a sincere thank you for your help in designing the experimental study and for your statistical consultancy during the data analyses phase of this research. I am also extremely grateful for providing me the opportunity to participate in your statistics course on the use of SAS, it was truly a life changing statistical experience!

I would like to thank to the Animal Welfare Program of University of British Columbia, University of British Columbia Graduate studies and James A. Shelford Memorial Scholarship award for providing various fellowships, scholarships, and award to support my study.

Thank you to all the staff and students at the Animal Welfare Program, both in Vancouver and at the UBC Dairy Education and Research center in Agassiz. I could not have completed my research trial without the help of the students on the dairy centre. Great thanks go to Grazyne Tresoldi and Lori Vickers for their encouragement and for their generous offer to take over the data collection when I was in need of an occasional break away from the dairy centre. Grazyne thank you also for being willing to be one of the inter-observer team.

The greatest thanks go out to my family. Thanks to my wife, Rahel, for your extraordinary love, support and encouragement throughout my life. Your constant love, patience and encouragement boosted me to focus on my thesis work. Your patience when I take your time and get upset and your encouragement to persevere when I doubted myself make the accomplishment really to happen. Many thanks also to my little son, Ebenezer, for giving me a big smile when I arrive home from work and for being a playful boy. Your big smile and being constantly playful with me and with your Mom was truly the best stress relieving environment at my home. I am proud of you, keep on these wonderful jobs.

Chapter 1: General Introduction

1.1 Dairy cattle industry and cow welfare

In the last two decades, North American dairy farming has become increasingly intensive. For example, from 1993 to 2011, the number of Canadian dairy farms and cows declined by 56.6% and 21.9% respectively, while milk production increased by 14.5% and average herd size increased from 53.3 to 80.6 (CDIC, 2011).

Free-stall and tie-stall barns are the two widely used cow housing systems used by Canadian dairy farmers, accounting 23% and 75% respectively. In British Columbia, free-stall barns are the most common, accounting for 91%, followed by 6 % tie-stall and 3% of farms using the robotic system (CDIC, 2011). Compared to tie-stall, cow housing in free-stalls reduces producer labour cost, but also provides cows the freedom to exercise and to perform social behaviour (Stookey, 1994).

Parallel to industry intensification, animal welfare has emerged as a growing research area greatly influenced by social and ethical concerns (Fraser, 2008). Animal welfare research has focused on three main themes: the animal's health and biological functioning, the animal's affective states, and the animal's ability to live a "natural" life (Fraser et al., 1997). These areas of concern are also captured in the Five Freedoms: freedom from hunger, thirst and malnutrition, freedom from physical and thermal discomfort, freedom from pain, injury and disease, freedom from fear and distress, and freedom to express normal patterns of behaviour (OIE, 2011).

1.2 Cow social behaviour and free-stall housing

As cows are social animals, the free-stall system benefits cows with freedom to exercise and to perform social and other natural behaviour (Stookey, 1994). Within this system, however, the quality of life of cows is dependent on the specific features of the interaction between the cow and its environment. Features such as stall design, flooring type, space and design of the feed bunk, stocking densities, regrouping management and the behaviour of handlers have important effects on cow welfare (Rushen et al., 2008).

1.2.1 Stocking density

In free-stall barns, overstocking refers to housing more cows than the number of stalls in a pen and/or providing less feed bunk space than the recommended space of 0.6 m per cow (Grant and Albright, 2001). Previous work has shown that overstocking increases competition and displacement of cows by group-mates in stalls or at the feed bunk (DeVries and von Keyserlingk, 2006; Fregonesi et al., 2007a; Hill et al., 2009; Proudfoot et al., 2009). As well as increasing social aggression and competition for resources among pen-mates, high stocking density at the feed bunk is also accompanied by a decrease in feeding time (DeVries et al., 2004; Huzzey et al., 2006) and increased feeding rate (Olofsson, 1999). Similarly high stocking density at the lying area can also result in decreased lying time, for example, relative to 100% stocking density (i.e., 1 stall and 0.6 m of feed bunk space per cow), lying and ruminating time of cows was decreased when stocking density at the stall increased to 109, 120, 133, and 150% (Fregonesi et al., 2007a).

Previous work also reported that competitive interactions and displacements were much higher at the feed bunk than at the lying stalls (Val-Laillet et al., 2009). This suggests that the design of the feeding area is especially important for minimizing competition and displacements, for example, by providing more space per cow (DeVries et al., 2004; Huzzey et al., 2006), or by adding physical barriers between feeding positions (DeVries and von Keyserlingk, 2006).

1.2.2 Bedding type and depth

Poor bedding type and inadequate depth of bedding are associated with poor cow comfort as indicated by reduced lying time (Tucker and Weary, 2004). Lying time has also been shown to decrease when sand bedding levels in free-stalls were not maintained (Drissler et al., 2005). Similarly, cows housed in tie-stall barns spent more time lying when the depth of both straw and sawdust bedding was increased (Tucker et al., 2009). Cows also spent more time lying in the stall bedded with dry sawdust compared to wet sawdust (Fregonesi et al., 2007b; Reich et al., 2010) and spent more time perching on wet compared to dry sawdust bedding (Fregonesi et al., 2007b). Dairy calves also showed a clear preference for sawdust bedding compared to concrete flooring (Camiloti et al., 2012), and calves lay for a longer time on drier sawdust bedding than on wet sawdust Camiloti et al., 2012).

1.2.3 Regrouping

Regrouping of unfamiliar animals often increases agonistic behaviour of farm animals including cows (Hasegawa et al., 1997; von Keyserlingk et al., 2008; Neisen et al., 2009), pigs (Hyane and Gonyou, 2006; Samarakone and Gonyou, 2008), horses (Christensen et al., 2011), sheep (Sevi et al., 2001) and goats (Fernández et al., 2007; Andersen et al., 2008). This increased agonistic behaviour commonly leads to stress-related behavioural and physiological reactions (González et al., 2003; Miranda-de la Lama et al. 2012), social instability (Andersen et al.2008), reduced feed intake, lying time, milk production (von Keyserlingk et al., 2008), and body weight (Nakanishi et al., 1993). In goats, regrouping increased escape behaviour and concentration of plasma and metabolites cortisol (Andersen et al., 2008; Patt et al., 2012).

Problems associated with regrouping are greater for introduced animals than resident ones (Bøe and Færevik, 2003; Schirmann et al., 2011), and for heifers introduced singly than those introduced in a pair or a group (O'Connell et al., 2008). Introducing cows into a new social group as a pair or group of three is recommended because the presence of a companion may have a calming influence (Sato and Tarumizu, 1993; Takeda et al., 2000), allows for the provision of social facilitation (Dindo et al., 2009), reduces social tension (Boissy et al., 2007), and generally lowers the stress response of animals to aversive situations (Boissy et al., 2007).

Studies have shown that heifers introduced in pairs or group of three had fewer agonistic interactions (Bøe and Færevik, 2003; Niesen et al., 2009), and integrated into a herd more easily (Gygax et al., 2009). Similar, O'Connell et al. (2008) also found increased activity when heifers were introduced in pairs rather than individually into established social groups. The authors interpreted the increased activity as a reflection of reduced fearfulness resulting from the presence of companion.

In contrast to the above advantages, Menke et al. (2000) found a higher rate of agonistic interaction after the integration of three heifers rather than a single heifer, suggesting that mixing of multiple group-mates into a new social group may also increase behavioural disruption among resident cows.

Social licking behaviour may play an important role in positive social facilitation or calming if an individual is mixed in the presence of familiar group-mates. Phillips and Rind (2001), noting an increase of social licking by primiparous cows after mixing with multiparous cows, considered the behaviour to be a means to reduce social stress. In contrast, in stable social groups, a decrease of social licking was reported to accompany an increase of displacements at the feedbunk due to overstocking (Val-Laillet et al., 2009), and due to regrouping when cows were introduced singly into a new social group of 11 cows (von Keyserlingk et al., 2008). Researchers have recommended further studies on the relationship between social licking and social competition after regrouping (Val-Laillet et al., 2009).

1.3 Agonistic social behaviour and social instability after regrouping

After mixing, cows typically have a period of social instability while dominance relations are established (Kondo and Hurnik, 1990). The degree and duration of social instability among members of a group may vary depending on a variety of factors (Bøe and Færevik, 2003). Animal factors include breed, age, sex, temperament and the previous mixing experience of individuals. Environmental factors include feed availability (feed quality, quantity and accessibility), and space available per animal (Bøe and Færevik, 2003). A lesser degree of agonistic interactions, plus earlier re-establishment of social stability after regrouping, are usually assumed to indicate good welfare (Kondo and Hurnik, 1990).

One method of assessing social stability or establishment of dominance relations after regrouping is to compare behaviour after regrouping with a baseline level (before regrouping) (Bøe and Færevik, 2003). Studies indicate that social instability of regrouped cows lasts for 3 to 7 days (reviewed by Grant and Albright, 2001), and that social and locomotion behaviours are altered for 5 to 15 days (Bøe and Færevik, 2003). In studies at the UBC Dairy Education and Research Centre, for example, agonistic interaction of mid-lactation cows returned to its baseline levels at 3 days after regrouping (von Keyserlingk et al., 2008), and feeding rate and rumination time of prepartum cows returned to baseline levels at 2 days after regrouping (Schirmann et al., 2011).

A second method of assessing social stability, proposed by Kondo and Hurnik (1990), is to investigate the proportion of non-physical to physical agonistic behaviours. According to Kondo and Hurnik (1990), social stability is established when non-physical agonistic interactions predominate over physical agonistic interactions, and/or the ratio of physical to non-physical interactions remains comparatively stable. However, this has rarely been studied.

1.4 Individual variation

Behavioural and physiological variation has been reported among conspecific individuals in response to the same stressors (Koolhass et al., 1999; Carere et al., 2010). This individual variation is likely due to differences in some combination of early experience (extrinsic) and biological variables (intrinsic) (Brouček et al., 2008; Koolhaas, 2008). For example, behavioural variation among individuals has been associated with age, size and body weight differences (Brouns and Edwards, 1994), dam parity, gender and sire lineage of the individual (Brouček et al., 2011), and early life experience including social housing and mixing (Bøe and Færevik, 2003).

Individual variation in response to a stressor can be either short-lived or stable (consistent) over time and across situations (Jensen et al., 1995). Individual differences that are consistent over time and across situations have been called "personality" (Forkman et al., 1995; Carere, et al., 2010), "temperament" (Réale et al., 2007), "behavioural syndrome" (Sih et al., 2004) or "coping styles" (Wechsler, 1995; Koolhaas et al., 1999). These are seen as alterative response patterns to a stressor (Koolhaas et al., 2010) consisting of a correlated set of behavioural and physiological characteristics (Coppens et al., 2010) or a consistent emotional and behavioural response of animals to different situations (Réale et al., 2007).

Based on the behaviour of mice in social conflict, Henry and Stevens (1977) suggested the presence of two distinct physiological response patterns. Later, this thinking was expanded by Benus et al. (1987) when they described two coping styles (active and passive) using two genetic lines of aggressiveness, whereby, male mice were selected either for short attack latency (SAL) or for long attack latency (LAL). In social conflict, mice of the SAL strain showed high levels of aggression, while mice of the LAL strain showed low levels of aggression. Studies on mice and rodents have generated the idea that individuals showing high levels of aggression represent the active coping style which is related to activation of the fight-flight response described by Cannon (1929). In contrast, individuals showing low levels of aggression represent the passive coping style which involves predominantly the conservation-withdrawal response (Engel and Schmale, 1972).

Further research has suggested a wide range of correlated responses. High aggressive or active coping style has been associated with low immobility, low flexibility, short attack latency, high defensive burying, low HPA-axis reactivity and high reactivity of the sympathetic nervous system (Koolhaas et al., 1999; Koolhaas, 2010). In contrast, low aggressive or passive coping animals show high immobility, high flexibility, long attack latency, low defensive burying, high HPA-axis reactivity and high parasympathetic reactivity in response to stressful situations.

According to Koolhaas et al. (2010) the difference in flexibility between the two coping styles of individuals is also related to how individuals adapt to the environment. Active-coping individuals tend to rely on previous experience or on rather rigid internally organized responses and appear better adapted to stable rather than to dynamic social conditions. Alternatively passive-coping animals tend to rely on more detailed appraisal of the current environment and do better in unpredictable and unstable social conditions.

In studies of rodents and pigs, aggressiveness is often measured in a ‘resident-intruder’ test, where a conspecific intruder is introduced into the home pen of the resident, and the speed or level of attack by the resident is recorded (Kemble, 1993; Erhard and Mendle, 1997). After mixing or regrouping into new social groups, high-aggressive individuals in the resident-intruder test were injured more often, but became more dominant (Fokkema et al., 1995). In mice, high-aggressive animals generally trigger aggression at mixing, but most often lose the fight to the resident (Hilakivi-Clarke and Lister, 1992). Similarly, high-aggressive pigs in a resident-intruder test showed a high level of aggression, more injuries and slower group integration after mixing than low-aggressive pigs, and in groups containing both high- and-low aggressive pigs, the highly aggressive pigs became dominant (Erhard et al., 1997).

Recently individual variation in aggression or coping style has been linked to life history (Boon et al., 2007; Biro and Stamps 2008; Réale et al., 2009), metabolic rate (Martins et al., 2011) and immunity or oxidative status (Koolhaas, 2008; Réale et al., 2010; Costantini et al., 2012). In sheep, docile (low-aggressive) males tended to have delayed reproduction and greater longevity (Réale et al., 2009), whereas bold (high-aggressive) ewes tended to show earlier age at first reproduction, and increased weaning success (Réale, et al., 2000). Furthermore, high-aggressive pigs showed faster growth rate and produced piglets with heavier birth weight at their first parturition, compared to low-aggressive pigs (Mendl et al., 1992).

In humans, oxidative stress is recognized as one of the factors affecting the progression of disease, ageing and life span (Salmon et al., 2010). Recent animal studies have also reported a link between individual differences and oxidative stress physiology in mice (Costantini et al., 2008; Rammal et al., 2010), greenfinches – *Carduelis chloris (*Herborn et al., 2011), and in wild alpine marmots – *Marmota marmota* (Costantini et al., 2012). In general, a higher baseline non-enzymatic antioxidant capacity was found in low-aggressive animals than in high-aggressive ones, and conversely, high-aggressive animals tend to have higher levels of reactive oxygen species (Rammal et al., 2010).

Individual variation is documented widely across many species; for example, in rats (Benus et al., 1987), non-human primates (Suomi, 1997), birds and laying hens (Verbeek, 1999; Korte et al., 1999) and in pigs (Hessing et al., 1993, 1994; Forkman et al., 1995; Jensen et al., 1995; Erhard and Mendl, 1997; Ruis et al., 2000; D'Eath and Burn, 2002; Geverink et al., 2003; Bolhuis et al., 2004). However, in dairy cows, the existence of such individual variation and its relationship to coping styles and other welfare indicators has been documented in relatively few studies (Van Reenen et al., 2002; Van Reenen et al., 2005).

1.5 Problem and objectives

A previous study of regrouping at the UBC Dairy Education and Research Centre has shown that mixing cows singly into new social groups results in high aggression and competition until new dominance relations are established, combined with reduced lying and milk production (von Keyserlingk et al., 2008). In that study, mid-lactation cows were introduced individually into a new social group of 11 cows.

In this study, we repeated the main features of the previous work, but we introduced cows in pairs rather than singly, and we collected more extensive information on social behaviour and self-licking. The objectives were: (i) to investigate how regrouping affects social behaviour, self-licking and milk production of cows introduced in pairs into a new social group, (ii) to determine the relationship between individual milk production and behavioural responses to regrouping, and (iii) to compare the milk production response to regrouping of introduced cows vs. resident cows. In addition, in order to explore the role of individual differences in regrouping, we used observations of aggressiveness before regrouping to: (i) investigate whether individuals show consistent variation in aggression before and after regrouping, and (ii) see how individual variation in aggression is associated with social licking, milk production, age at first calving and body weight.

Chapter 2: Effect of Regrouping on Social Behaviours, Self-licking and Milk Production of Mid-Lactation Dairy Cows Introduced in Pairs

2.1 Introduction

In modern dairy farms with large herd size, dairy cows are commonly reared in free-stall housing system. Cows in a free-stall have greater freedom for movement and exercise, and have more opportunity to perform social interactions (Stookey, 1994). However, for management purposes, cows in free-stalls are often regrouped with unfamiliar herd-mates (von Keyserlingk et al., 2008), and this regrouping can induce stress-related behavioural and physiological reactions (Miranda-de la Lama et al., 2012), increase aggressive interactions (Bøe and Færevik, 2003) and decrease feeding behaviour (von Keyserlingk et al., 2008).

There is also some evidence that regrouping is much more stressful for introduced animals than resident animals. For example, on the day of regrouping, feed intake of introduced cows declined by 9% compared to baseline whereas that of home resident cows did not change (Schirmann et al., 2011). Furthermore, an elevated concentration of cortisol metabolites and shorter feeding time was reported in introduced goats compared to resident goats (Patt et al., 2012).

A previous study showed the effect of regrouping on social behaviour and milk production of cows mixed individually into a new social group of 11 cows (von Keyserlingk et al., 2008). However, there is evidence that the presence of a familiar animal can mitigate the negative effects associated with the new social situation (Boissy et al., 2007; O'Connell et al., 2008; Dindo et al., 2009; Neisen et al., 2009). The following study, therefore, tested the response of cows introduced in pairs into new social groups. It also included more detailed observations on social and other behaviours.

The objectives were: (i) to investigate the effect of regrouping on social behaviour, self-licking and milk production of mid-lactation cows introduced in pairs into a new social group, (ii) to determine the relationship between the social behaviour response to regrouping and milk production of the introduced cows, and (iii) to compare the milk production response to regrouping of introduced cows vs. resident cows.

2.2 Materials and methods

2.2.1 Animals, housing and management

A total of 24 mid-lactation Holstein cows were used. They included 14 "focal" or "moved" cows (those whose behaviour was studied) and 10 "filler" or "resident" cows that made up the group into which the focal cows were introduced. Both selected "focal" and "filler" cows were healthy and confirmed pregnant. Focal cows (6 primiparous, 8 multiparous) ranged from parity 1 to 4 (mean ± SD: 1.9 ± 1.0) with days in milk (DIM) of 121-228 (193 ± 29), body weight (BW) of 620-810 kg (690 ± 59), and 305-days adjusted milk yield of 8,160-14,330 kg (11,896±1,711). Filler cows (3 primiparous, 7 multiparous) ranged from parity 1 to 4 (3.1 ± 1.4) with DIM of 148-228 (198 ± 27), BW of 500-760 kg (678 ± 75) and 305-days adjusted milk yield of 10,810-14,220 kg (12,673 ± 1,234). Data were collected from July to mid-September 2011 at The University of British Columbia Dairy Education and Research Centre, in Agassiz, British Columbia, Canada.

Before regrouping, focal and filler cows were housed as 2 separate groups, each in a free-stall pen 7.2 by 13.5 m equipped with 12 stalls configured in 3 rows (Fig. 2.1). Stalls were bedded with river washed sand and were raked 2 times daily when cows were away from the pen for milking. Bedding was replaced with fresh sand roughly once in 2 weeks. Before and after regrouping, cows in both pens were kept at 100% stocking density with one stall and 60 cm space at the feed bunk per cow. The "baseline pen" housed the focal cows before regrouping and the "regrouping pen" housed the filler cows. The two pens were separated by 30 m to prevent social contact between the two groups.

Cows were managed according to the guidelines set by the Canadian Council on Animal Care (CCAC, 2009), and were given ad libitum access to a Total Mixed Ration (TMR) for high-producing cows. Fresh feed was delivered twice daily at about 0600 and 1500h. Fresh water was available ad libitum from self-filling bins that were cleaned twice a day when cows were in the milking palour. Experimental cows were milked twice daily at about 0830 - 0900, and 1700 - 1730. Feed was pushed close to the cows usually 3 times daily at roughly 1100, 1900 and 2200. Manure was cleaned from the alley by automatic scraper 7 to 8 times per day.

2.2.2 Experimental design

The cows were chosen from the herd based on convenience. Numbers from 1 to 14 were assigned to each focal cow by a randomized procedure. Numbers were marked by hair dye on the animal's sides. Allocation of cows into replicates was done according the assigned number with 1 and 2 in the first replicate, and so on.

Before experimental regrouping started, each pen contained 12 cows, and cows in each pen (group) were stabilized for 7 days. In replicate 1, after 2 days baseline behavioural monitoring on the focal cows (e.g. cow 1 and 2) in the baseline pen, the 2 cows were moved to the regrouping pen at roughly 09:00 and the same behaviours recorded for an additional 5 days. At the same time 2 cows from the filler group were moved out of the filler group to the farm and another 2 focal cows (e.g. cow 13 and 14) from the dairy centre were added to the baseline pen.

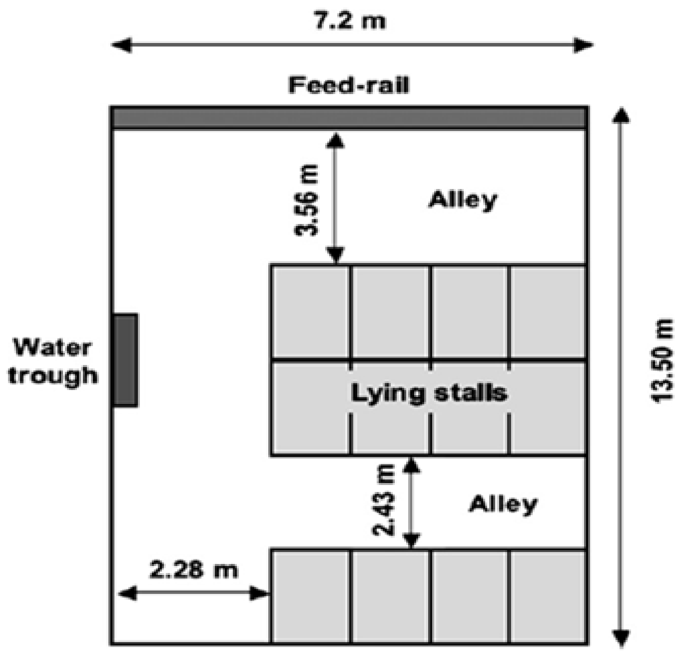


Figure 2. 1 Schematic diagram of the Experimental home pen for 12 cows

In replicate 2, focal cows 3 and 4 were monitored for baseline behaviour in the baseline pen and then moved to the regrouping pen where observation continued for 5 days. At the same time, focal cows 1 and 2 were returned to baseline pen. The same reshuffling pattern was repeated in the remaining replications by moving a new pair of focal cows to the regrouping pen, and in exchange, the 2 previously monitored focal cows in the regrouping pen were returned to the baseline pen. This maintained 100 % stocking density in both pens.

2.2.3 Data collection

Behaviour was observed for the 2 selected focal cows in each of 7 replications during 7 days (2 days before regrouping and 5 days after). To fit into farm routines, behavioural observations were carried out in 5 1-hour observation periods beginning at approximately 06:30, 09:00, 13:00, 15:30

and 18:30. Cows were regrouped immediately after milking at about 09:00 i.e. roughly 3h after fresh feed delivery (at 06:00).

Behavioural recording included all instances of physical and non-physical agonistic contacts and displacement, frequency and duration of social licking initiated and/or received by focal cows, together with self-licking, throughout the entire pen. Behaviours were recorded individually for each focal cow in a replicate by direct observation of a single observer using continuous recording method according to the definitions in Table 2.1. During the experiment, milk yields of focal and filler cows were automatically recorded at each milking.

2.2.4 Data analysis

The pair of cows or replicate was treated as the experimental unit (n=7). Individual records of each cow in a replication were averaged to represent the experimental unit value in the analysis. As cows were regrouped at about 09:00, to keep the time of observation consistent, the observational day was considered to begin at 09:00 and end at just before milking the next morning at 06:30. The 5 h of observation per day for each cow were summed, and daily results were reported as values per 5 h. Finally, data were summarized by replication and day. Given that the experimental day began at 9:00, this provided 5 days of complete data (i.e. 1 day before regrouping and 4 days after regrouping). Replication was included in the model, but was not significantly different.

To establish inter-observer reliability, data were collected in 10 sessions by 2 trained observers and correlations were computed for each behavioural category. The coefficient of determination (R^2) between the 2 observers was 99, 98, 92, 85 and 70% for total physical agonistic contacts, non-physical agonistic contacts, displacement, social licking and self-licking, respectively.

Because behavioural measurements were repeated on cows (i.e. before and after regrouping), data were analyzed by the PROC MIXED procedure in SAS (2011, version 9.3) for repeated measures ANOVA. Estimates of least square means and standard errors were generated for each variable using the LSMEANS statement. The contrast statement was used to test the effect of regrouping on the parameters by comparing each values on the day of regrouping (d 0) and after the day of regrouping (d +1, d +2, d + 3) against the value on the day before regrouping or baseline (d -1).

Table 2. 1 Definition of behavioural categories

Social behaviour	Description
Agonistic physical contacts	Physical contacts including head-to-head, head-to-neck, head-to-body and body-to-body made while animals were competing for resources, excluding physical contact that occurred before, during or after social licking.
Agonistic non-physical contacts (threats)	Activities judged to be threatening, including head swing directed toward another cow, chasing of a cow without contact, and eye-to-eye contacts that were visible to the observer causing displacements.
Displacement	Withdrawal of one cow from another including withdrawal of the head from the feed bunk rail, and withdrawal from a lying stall or position in the alleyway, after any physical or non-physical agonistic contacts from another cow. The displacement was scored as a physical displacement if it followed physical agonistic contact or non-physical displacement if it followed non-physical agonistic contact. A cow was scored as losing the displacement if it withdrew from another cow and as winning the displacement if another cow withdrew from it
Social licking	Repeated back-and-forth licking by a cow using the tongue on body parts of another cow except the anal area (Fraser and Broom, 1990). Separate social licking events were scored when the actor cow stopped licking for 15 sec or any time the recipient cow became the actor.
Self-licking	Licking by a cow of her own body

To test for normality and the presence of outliers, Proc Mixed Model was used to generate residuals for each variable; these residuals were then screened for normality and outliers using the Proc Univariate Procedure of SAS (2011, version 9.3). The normality assumption was met for all variables. However, outliers were detected for social licking and milk yield. Thus, the effects of outliers on the LS means, standard error and p-value were examined by repeating the analysis with and without the outliers. As outliers for social licking had no major effect on the estimates, the LS means, standard error and p-values are presented without excluding the outliers, but 2 outliers of milk yield to regrouping were deleted. Before records of individual cows within a replicate were averaged, parity (primiparous vs multiparous) was tested as a fixed effect, but was found to be not significant and was therefore excluded from the final analysis.

Proc Regression Procedure in SAS was used to examine the relationship of milk yield with the amount of agonistic and non-agonistic social behaviour initiated and received. Daily milk yield records of each cow on the day and 3 days after regrouping were used as the dependent variable. Records of social behaviour of cows on the same measurement days were used as independent variables. Milk yield before regrouping was used as a covariate.

Initially, for each cow, the percentage of social licking events (the number of social licking between pair focal cows divided by the total social licking of focal cows among all cows in the pen multiplied by 100) performed between the 2 focal cows was calculated for the day before, the day of, and the day after regrouping. Data were then summarized by replication. Proc GLM was then used to test whether the percentages differed between the two time periods.

2.3 Results

2.3.1 Agonistic contacts and displacements

The mean number of physical agonistic contacts and physical displacements increased sharply on the day of regrouping (d 0) and then declined over d +1 to d +3 (Fig. 2.2). In contrast the mean number of non-physical agonistic contacts and non-physical displacements showed much less change over the days observed in this study. As a result, physical contacts and displacements greatly out-numbered the non-physical on d 0, but the number of physical and non-physical events was quite similar by d +3 (Fig. 2.2).

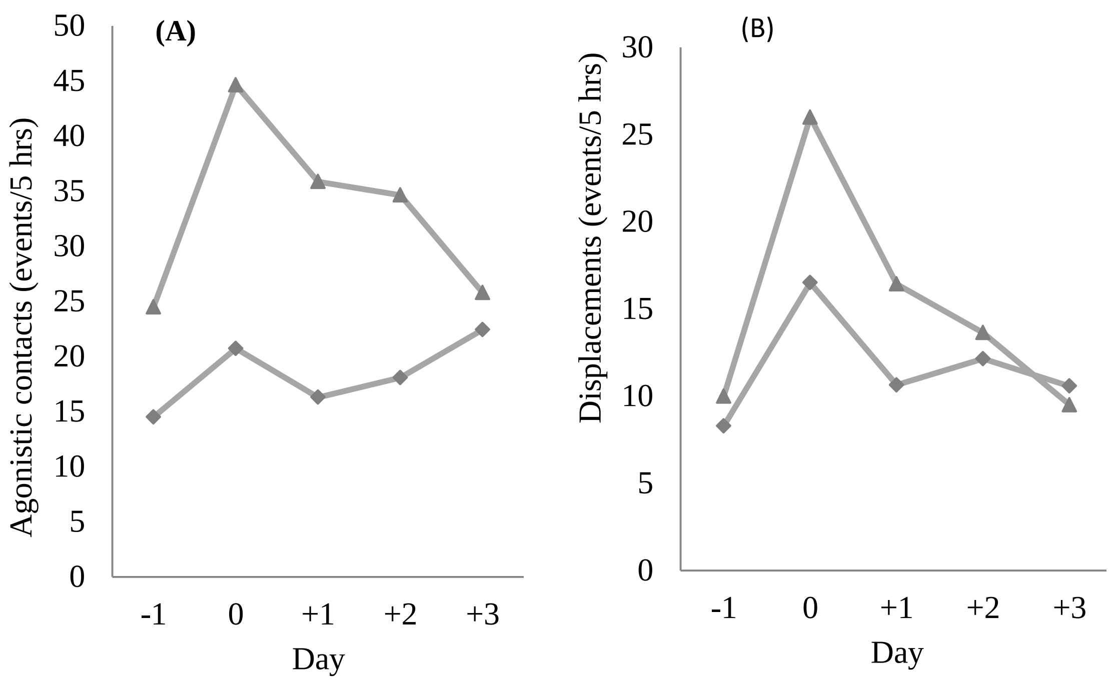


Figure 2. 2 Agonistic behaviour14 focal cows introduced into social group in pairs (n=7) before the day of regrouping (d -1), on the day of (d 0) and the days after of regrouping (d +1, d +2, d +3). Results show physical (triangles) and non-physical (diamonds), instances of agonistic contacts (A) and displacements (B). Data are based on 5 h of observations per day.

After regrouping, total agonistic contacts increased on d 0 ($P < 0.01$) and tended to decline on subsequent days (Table 2.2). The result was explained entirely by an increase in agonistic contacts received by the focal cows ($P < 0.01$), as there was no change in agonistic contacts initiated by the focal animals ($P > 0.05$).

After regrouping, focal cows were displaced by others more often up to d +2 than before regrouping (Table 2.2), with the largest occurrence on d 0 ($P < 0.001$). Focal cows displaced others more often on d 0 ($P < 0.05$), but not on the subsequent regrouping days ($P > 0.05$).

2.3.2 Social and self-licking

After regrouping, social licking events were not different from the baseline day ($P > 0.05$; Table 2.2), although events tended to decrease on d 0 ($P = 0.09$). After regrouping, the duration of social licking was lower than baseline on 3 of the 4 days ($P < 0.05$; Table 2.2). Self-licking by focal cows increased on the d 0 ($P < 0.05$), but not on subsequent days ($P > 0.05$).

Before regrouping, the proportion of social licking events performed between the pair of focal cows was 10 ± 9 %. After regrouping, including the day of regrouping, the proportion increased to 38 ± 9 % ($P < 0.05$).

2.3.3 Milk production

Before regrouping, milk production (kg/d) was 32.1 ± 1.4 and 33.5 ± 1.2 for focal (moved) cows and filler (resident) cow, respectively, and it did not differ between the focal (moved) and filler (resident) cows ($P > 0.05$, Table 2.3). After regrouping, milk production of focal cows decreased on average by 1.5 kg on the day of regrouping, but milk production on the day of regrouping and on subsequent days was not different from baseline ($P > 0.05$). Milk production of resident cows showed no differences in response to cows being introduced into the group (Table 2.3). Focal cows produced less milk than resident cows on d 0 and d +1 ($P < 0.05$, Table 2.3).

Regression analysis showed that milk production of focal cows after regrouping had a negative linear relation to total agonistic contacts received ($P = 0.01$) and to displacements lost ($P = 0.03$), but there was no relationship with other social behaviours ($P > 0.05$, Table 2.4).

Table 2. 2 Least Square means (±SE) of agonistic contacts (physical and non-physical combined), displacement, social licking events, social licking duration and self-licking events on d -1 (before regrouping or baseline), d 0 (on the day of regrouping) and d +1, d +2 and d +3 (after regrouping) for mid-lactation Holstein focal cows introduced in pairs (n=7) into social group of filler cows, and cows observed 5 h per day.

	Time period relative to regrouping				
Parameter	Baseline (d -1)	d 0	d +1	d +2	d +3
Agonistic contacts (events/d)[1]					
Total	39.0 ± 5.2	65.4 ± 5.2**	53.9 ± 5.2*	52.7 ± 5.2*	48.2 ± 5.2
Initiated	20.6 ± 4.6	24.3 ± 4.6	14.6 ± 4.6	17.9 ± 4.6	17.6 ± 4.6
Received	18.4 ± 5.0	41.1 ± 5.0***	39.4 ± 5.0**	34.8 ± 5.0**	30.6 ± 5.0**
Displacement (events/d)					
Total	18.3 ± 3.3	42.5 ± 3.3***	27.1 ± 3.3*	25.8 ± 3.3	20.1± 3.3
Won	9.5 ± 2.3	15.6 ± 2.3*	7.6 ± 2.3	9.1 ± 2.3	6.5 ± 2.3
Lost	8.8 ± 3.7	26.9 ± 3.7***	19.4 ± 3.7*	16.7 ± 3.7*	13.6 ± 3.7
Social licking (events/d)					
Total	2.1 ± 0.5	1.1 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	2.6 ± 0.5
Initiated	1.2 ± 0.4	0.6 ± 0.4	0.9 ± 0.4	0.7 ± 0.4	1.4 ± 0.4
Received	0.9 ± 0.3	0.4 ±0.3	0.8 ± 0.3	1.0 ± 0.3	1.1 ± 0.3
Social licking duration (min/d)	5.6 ± 1.1	2.5 ± 1.3*	4.5 ± 1.2	1.5 ± 1.0*	2.2 ± 1.1*
Self-licking (events/d)	6.6 ± 1.4	10.3 ± 1.4*	6.6 ± 1.4	8.6 ± 1.4	7.1 ± 1.4

Significantly different from d -1: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; [1]Data/d indicates data collected during 5 h of observation on each day

Table 2. 3 Least Squares means (±SE) of milk production (kg/d) for introduced 14 focal cows (moved) and 10 filler cows (resident) on the day (d 0), after (d +1, d +2, d +3) of regrouping compared to the day before regrouping (d -1).

	Time period relative to regrouping*				
Cows	Baseline (d -1)	d 0	d +1	d +2	d +3
Focal cows	32.1 ± 1.6	30.6 ± 1.1	30.3 ± 1.0	31.4 ± 1.0	31.4 ± 1.6
Filler cows	33.5 ± 1.6	34.6 ± 1.1	33.8 ± 1.0	34.6 ± 1.0	33.9 ± 1.6
P-value**	0.55	0.02	0.03	0.06	0.29

*Milk production of the focal and filler cows was not different from baseline on the day of regrouping and on each of the 3 d following. **Statistical significance of the difference of focal cows vs. filler cows on each day.

Table 2. 4 Regression analysis of after regrouping milk yield (as dependent variable) of focal cows introduced in pairs (n=7) against independent variables of agonistic contacts (physical and non-physical combined), displacements (physical and non-physical combined) and social licking events, data (both milk yield and behaviours) recorded on d 0, d +1, d +2 and d +3.

	Regression of milk yield after regrouping		
Behaviour	slope ± SE (kg)	t-value	P
Agonistic contacts			
Initiated	0.02 ± 0.02	1.23	ns
Received	-0.03 ± 0.01	-2.28	0.03
Displacements			
Won	0.04 ± 0.03	1.35	ns
Lost	-0.04± 0.02	-2.14	0.04
Social licking events			
Initiated	0.11 ± 0.22	0.51	ns
Received	0.04 ± 0.26	0.17	ns

2.4 Discussion

In this study, the introduction of pairs of cows into an established group was followed by a large increase in displacements, especially on the day of regrouping, as has been commonly reported in earlier work (von Keyserlingk et al., 2008; Schirmann et al., 2011). However, by studying social behaviour in more detail, the present study has shown that the change involves a large increase in both physical agonistic contacts and physical displacements on the first day, with steady declines in both of these behaviours over the next 3 d. In contrast the non-physical agonistic contacts and non-physical displacements showed much less change over the same period. By the fourth day after regrouping (d +3), the ratio of physical to non-physical behaviour had returned to baseline levels, suggesting that social stability had been re-established according to the criteria of Kondo and Hurnik (1990). This finding is also in accord with the results reported by von Keyserlingk et al. (2008).

Before regrouping, the focal cows received and initiated roughly similar numbers of agonistic contacts, and they won as many displacements as they lost. This changed greatly after regrouping, and even by d +3 the focal cows continued to receive about twice as many agonistic contacts as they initiated and to lose twice as many displacements as they won. This suggests that even after social stability had been established, the introduced cows continued to be of lower social status than before regrouping. In accord with our result, introduced mice into a group tend to trigger aggression at mixing, but most often lose to the residents (Hilakivi-Clarke and Lister, 1992).

In previous work, mid-lactation dairy cows introduced singly into a new social group showed a large increase in displacements lost, a large decline in the amount of social licking, (especially social licking initiated, which remained depressed for 3 d i.e. on the day of regrouping and on the subsequent 2 d after regrouping), and a reduced milk yield on average by 3.7 kg, on the day of regrouping (von Keyserlingk et al., 2008). Although the present study was not an experimental comparison of introducing cows singly versus in pairs, the comparison of the results (cows introduced in pairs) with the earlier study (cow introduced singly) showed some similarities and some differences.

The increase in displacements lost was at least as great as in the previous study, and it remained elevated on d 0, d +1 and d +2. This suggests that the presence of a familiar partner did not have any obvious effect in preventing social aggression. In another regrouping study, Holstein heifers mixed as pairs into new social groups appeared to spent more time together, lay down more, produced greater milk components, and were more active than heifers mixed individually (O'Connell et al., 2008), suggesting that the presence of a familiar individual lowered the stress response to regrouping.

There is conflicting evidence for the effect of regrouping on social licking in cattle. Phillips and Rind (2001) found increased social licking after regrouping, but others found a decrease (von Keyserlingk et al., 2008). Results of the present show, on the day of regrouping (d 0), the average duration of social licking declined compared to baseline. On the same day (d 0) the number of social licking events tended to decline ($P = 0.09$) when agonistic contacts were highest and gradually returned to baseline levels by d +3 when social groups were relatively stable and aggression decreased. Similarly, Sato et al. (1993) found a difference between two herds of cattle in the duration of social licking; the difference was attributed partly to herd instability whereby cows in the more stable herd had longer duration of social licking. Thus, the current findings presented in this thesis are most consistent with view that social licking is a sign of social stability.

After regrouping, the present results also showed an increase in self-licking on the day of regrouping (d 0) compared to the baseline period. This increase of self-licking may be linked to stress arising as a consequence of regrouping, as there are several reports of self-licking in response to aversive situations. For example, an increase of self-licking was reported among birds exposed to social conflict (Manning and Dawkins, 1992), mammals exposed to novelty (Munksgaard et al., 1996; Herskin et al., 2004), rabbits housed in barren cages without a shelter or hay (Hansen and Berthelsen, 2000), and calves housed in restricted space (Ferrante et al., 1998) or housed individually rather than in groups (Kerr and Wood-Gush, 1987).

Whether or not social licking played a direct role is difficult to determine. However, the stress-reducing effect of a familiar partner may explain why focal cows in this study showed no reduction in milk production on the day of regrouping compared to the baseline level, unlike cows introduced singly (von Keyserlingk et al., 2008). Similarly, in previous studies, the beneficial effects of social licking in reducing social stress have been associated with positive correlation between being licked vs. milk production and weight gain (Napolitano et al., 2007). In other studies, social licking recipient cows also reported to have lower heart rate (Takeda et al., 2003; Sato and Tarumizu, 1993; Laister et al., 2011) and show behavioural changes such as stretching the licked part of the body, a slight raising of the tail, and half closing of the eyes (Sato et al., 1993). It has been argued that such physiological and behavioural changes are a clear indication of the role social licking plays in reducing social stress associated to positive affective state (Boissy et al., 2007).

Another possible reason for the difference in milk production results between the two studies, i.e. the present study and von Keyserlingk et al. (2008), could be due to differences in feeding behaviour arising from differences in timing of regrouping and fresh feed delivery between the two studies. Cows in the present study were regrouped immediately after milking at 09:00- roughly 3 h after cows had access to fresh feed, whereas cows in the von Keyserlingk et al (2008) were regrouped at 06:00 before fresh feed delivery. Previous research has shown that cows are highly motivated to access fresh feed (DeVries and von Keyserlingk, 2005) and the hours immediately following fresh feed delivery are associated with high displacement activity (DeVries et al., 2005) and reduced feeding time (DeVries et al. 2003). Given that the cows in the current study were able to consume fresh feed for 3 h before being regrouped the negative effects leading to decreased milk production on the day of regrouping compared to baseline of the introduced cows may have been mitigated. Moreover, milk production of the focal cows was lower compared to resident cows on each of the days following regrouping, suggesting that regrouping is more stressful for introduced cows than for resident cows, as shown clearly by the negative regression association individual milk yield of focal cows with agonistic contacts received and displacements lost. On the day of regrouping, dry matter intake of introduced prepartum cows reduced by 9% compared to the baseline, but resident prepartum cows not (Schirmann et al., 2011).

2.5 Conclusion

The present results suggest that regrouping of mid-lactation cows in pairs into a new social group increases agonistic contacts, displacement and self-licking behaviours, but decreases duration of social licking among newly introduced cows compared to baseline values. The milk production results suggest regrouping is more stressful for introduced cows than resident cows, especially for those that received the most agonistic contacts and were displaced most often. Mixing of mid-lactation cows in the presence of a companion may be beneficial, perhaps partly by allowing them to engage in more social licking activities that may reduce the social stress of mixing.

Chapter 3: Individual Variation in Aggression and Its Relationship with Social and Physiological Variables in Lactating Cows Regrouped in Pairs

3.1 Introduction

As noted in chapter 1, behavioural and physiological variation has been reported among conspecific individuals in response to stressors (Koolhaas et al., 1999; Carere et al. 2010). Some individual variation is believed to be stable or consistent over time and/or across situations (Jensen et al., 1995; Koolhaas et al., 1999), and is likely due to differences in some combination of biological factors and the early life experience of individuals (Brouček et al., 2008; Koolhaas, 2008). These consistent individual differences have been called "personality" (Forkman et al., 1995; Carere, et al., 2010), "temperament" (Réale et al., 2007), "behavioural syndrome" (Sih et al., 2004), or "coping styles" of individuals (Koolhaas et al., 1999).

The presence of two distinct and extreme physiological response patterns among individuals of the same species was first suggested by Henry and Stevens (1977). This thinking, combined with research on two genetic lines of mice selected based on their aggressiveness, was later expanded to describe two coping styles (Benus et al., 1987). Active-coping mice show high levels of aggression associated with the fight-flight response (Cannon, 1929); whereas, passive-coping mice show low levels of aggression and a conservation-withdrawal response to stressors (Engel and Schmale, 1972).

Although such individual variation is widely documented across many species, in dairy cows there has been limited research on consistent individual differences in aggressiveness and coping styles in relation to animal welfare (Van Reenen, et al., 2002). Knowledge of such individual variation could play a role in reducing social stress in situations such as social mixing and/or high stocking density. For example, mixing high-aggressive animals might impair their welfare, as high-aggressive animals are more likely to suffer from injuries (D'Eath, 2002; Haller et al., 2006) and possible distress after losing fights (Mendl, 1995; Boissy et al., 2007). In the conceptual framework developed by Réale et al. (2010) it was stated that low aggressiveness of individuals was associated with high levels of sociability and parental care, low growth rate, delayed reproduction and long longevity. In contrast, these authors stated that high aggressiveness was associated with low levels of sociability and parental care, faster growth rate, early reproduction and short longevity. However, there appears to be no scientific studies substantiating these proposed associations. The present study is thus the first attempt to investigate these associations.

The present study used data collected in Chapter 2 to address two further objectives: (i) to investigate whether individuals show consistent variation in aggression before and after regrouping, and (ii) to see how individual variation in aggression is associated with social licking, milk production, age at first calving and body weight of cows.

3.2 Materials and methods

3.2.1 Animals, housing and management

This study used the data from the 14 focal cows described in Chapter 2. Briefly, the cows were observed for 5 h per day on 2 days before regrouping and 5 days after being mixed in pairs into a new group of 10 cows. Each cow was weighed twice (i.e. during the stabilization period and again at the start of each replicate), and the records of each focal cow were averaged for analysis. Age at first calving (days) was retrieved from the breeding records of the dairy centre; and the 305-day Mature Equivalent (ME) milk production of each cow was calculated from days in milk using Dairy Comp 305 software (Comp 305, Valley Agriculture Software, Tulare, CA, US).

3.2.2 Statistical analysis

Individual differences in aggressiveness were identified based on the Displacement Index (DI) adapted from Galindo and Broom (2000) and Mendl et al. (1992). “Diplacement Index” is the number of times that an individual displaced other cows expressed as a percentage of the total displacements that involved the individual.

Specifically:

$$\text{Displacement Index} = \left(\frac{\text{number of times that an individual displaced other cows}}{\text{number of times that an individual displaced other cows} + \text{number of times than an individual was displaced by other cows}} \right) X100$$

$$\text{Initiated aggression Index} = \left(\frac{\text{number of agonistic contacts initiated by an individual}}{\text{number of agonistic contacts initiated to others} + \text{number of agonistic contacts received from other cows}} \right) X100$$

Each Index was calculated for each of the 14 focal cows for 2 periods: the 2 days of baseline observation before regrouping and the 5 days of observation after regrouping. Cows were classified into 3 groups based on the Displacement Index (DI) before regrouping using the criteria of Galindo and Broom (2000): high-aggressive (HA: DI ≥ 60), middle-aggressive (MA: 40 < DI < 60), and low-aggressive (LA: DI ≤ 40). Based on these criteria, the 14 cows consisted of 5 low-aggressive, 3 medium-aggressive and 6 high-aggressive cows. Aggressive cows during the baseline period were nearly always successful when they engaged in an aggressive interaction. However, when regrouped and subjected to a socially unstable situation they frequently initiated an aggressive interaction but did not necessarily win it. Thus, in addition to the Displacement Index, the Initiated Aggression Index (IAI) was also used to assess the aggressiveness consistency of individuals before and after regrouping. “Initiated Aggression Index” is the number of agonistic (considering both physical-and non-physical) contacts initiated by an individual as percentage of the total agonistic contacts that involved the individual. Each variable was examined for presence of outliers and normal distribution; one outlier was identified from the in the case milk yield and duration of social licking data and these data points were removed prior to the final analyses. Measures of individual consistency before and after regrouping (coefficient of determination, slope ± SE and P-value) were calculated for both the Displacement Index and Initiated Aggression Index using Proc Regression procedures of SAS (version 9.3). Following preliminary analyses it was determined that the inclusion of the 3 medium-aggressive cows had little effect on the estimates and thus these cows were excluded from analysis, leaving n=11 cows (i.e. 6 high-aggressive and 5 low-aggressive) in the final data to assess individual variation in aggression consistency and in the variance analysis of all other variables. The regression of the Displacement Index score after regrouping on body weight was analyzed in a similar way.

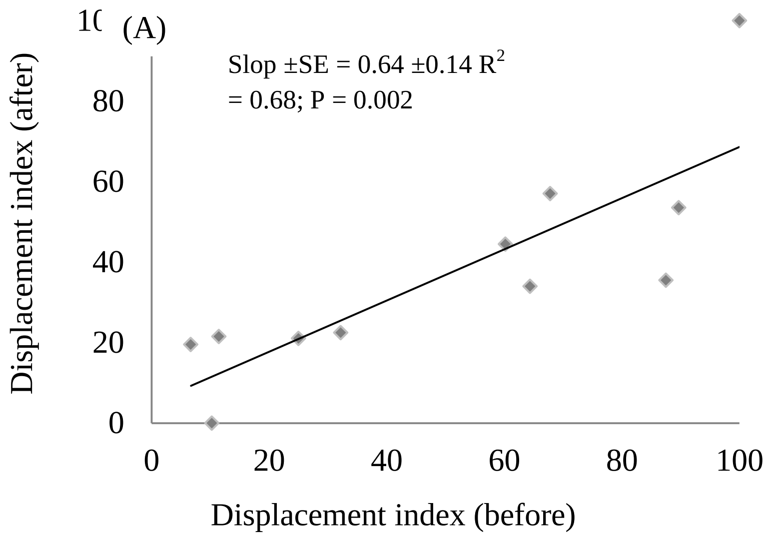
Mixed model was used to test individual difference between 6 high- and 5 low-aggressive cows after regrouping for agonistic contacts, displacements, social licking behaviour and milk yield variables. The aggressiveness category for each cow (high-aggressive vs. low-aggressive) was fitted as a fixed effect with two levels. Cows fitted as subject for repeated measures and pair was included in the model. Age at first calving was analyzed using GLM model. For the analysis of milk yield after regrouping, daily milk yield before regrouping was fitted as a covariate. Initial analysis of variance tested for an interaction between milk yield before regrouping and cow aggressiveness category; this interaction was not significant and therefore was dropped in the final analysis.

3.3 Results

3.3.1 Consistency of individual aggression variation

The Displacement Index after regrouping was strongly related to the Displacement Index before regrouping ($R^2 = 0.68$; $P = 0.002$, Fig 3.1A). Similarly, the Initiated Aggression Index after regrouping was strongly correlated to that Index before regrouping ($R^2 = 0.75$; $P < 0.001$; Fig 3.1B).

The Displacement Index after regrouping was closely related to the Initiated Aggression Index during the same period (R^2 = 0.96; P < 0.001; Fig 3.2A). There was no relationship between the Displacement Index and body weight (R^2 = 0.07; P > 0.05, Fig 3.2B). However, the heaviest focal cow won 100% of displacements, whereas the third-lightest focal cow failed to win any displacements (Fig 3.2B).



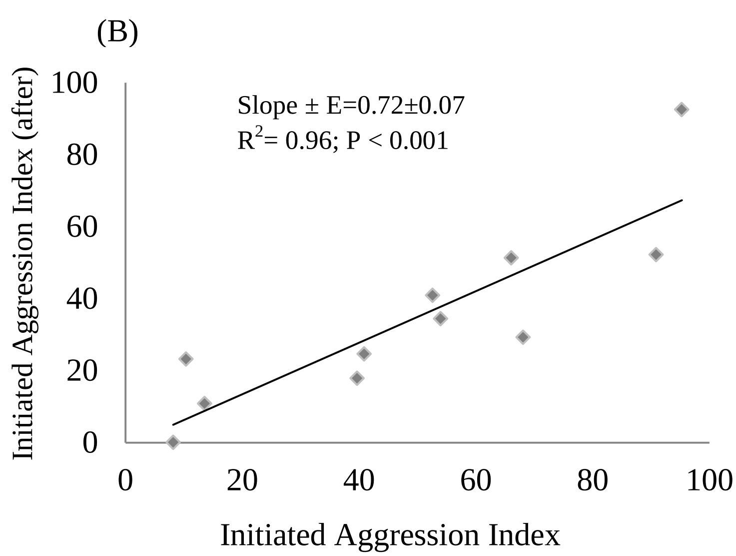


Figure 3. 1 Consistency of individual variation in the Displacement Index (A) and Initiated Aggression Index (B) before and after regrouping of mid-lactation Holstein cows (n=11).

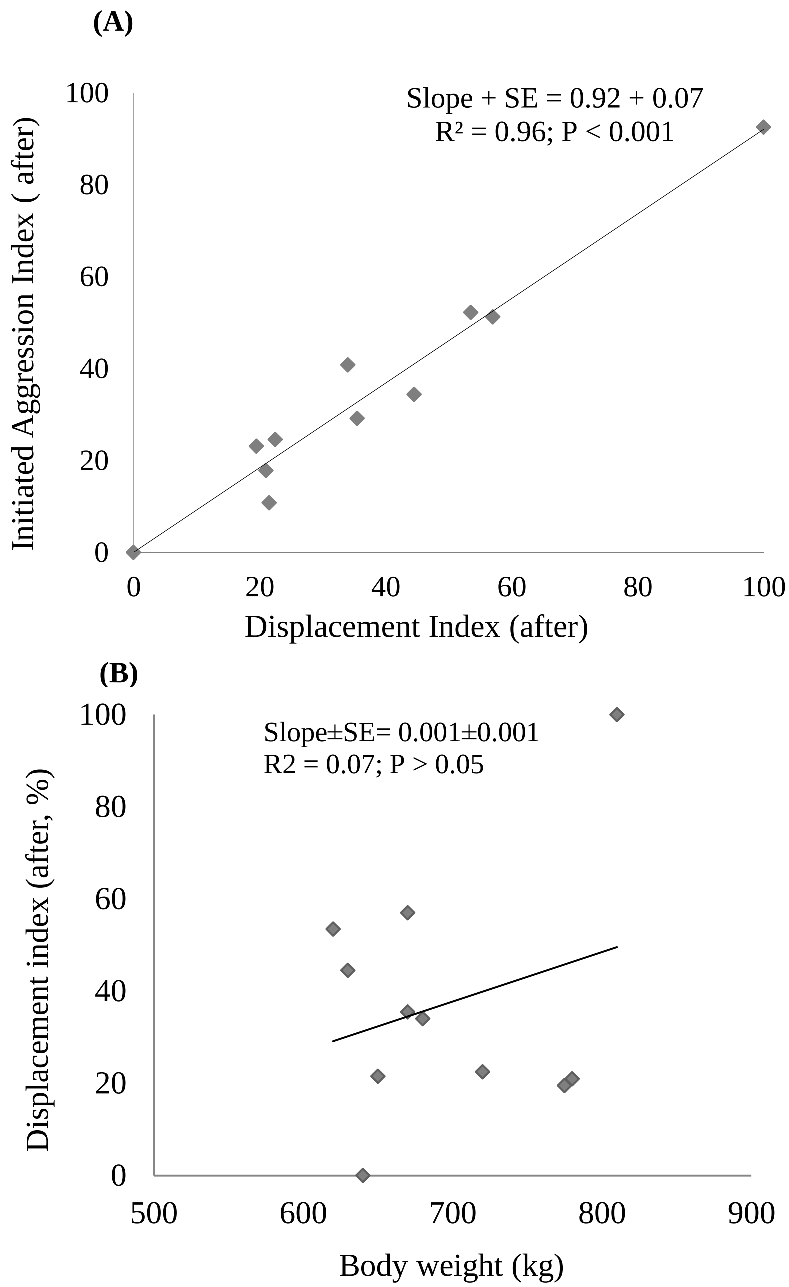


Figure 3. 2 Relationship between Displacement Index, Initiated Aggression (A) and between Displacement Index after regrouping and body weight (B) for mid-lactation Holstein cows (n=11).

3.3.2 Differences between high-aggressive and low-aggressive cows

After regrouping, high-aggressive cows more often initiated agonistic contacts ($P < 0.01$) and displaced other cows ($P < 0.01$) than low-aggressive cows (Table 3.1). High-aggressive cows also received far fewer agonistic contacts ($P < 0.001$) and were displaced less often by others ($P < 0.01$; Table 3.1). No differences between high-aggressive and low-aggressive cows were found for social licking events, or self-licking events ($P > 0.05$; Table 3.1).

After regrouping, there was no difference in milk production between high-aggressive (30.1 ± 1.1 kg/d) and low-aggressive cows (29.8 ± 1.3 kg/d, $P > 0.05$). However, projected 305-day mature equivalent (ME) milk production was higher for low-aggressive cows (12,928 ± 580 kg) than for high-aggressive cows (10,530 ± 530 kg; $P = 0.01$). Taking into consideration age at first calving, it was predicted that high-aggressive cows would be younger at first calving compared to low-aggressive cows. Contrary to our prediction, the present result of age at first calving was found (734 ± 9 days) for high-aggressive cows and (737 ± 9 days) for low-aggressive cows.

Table 3. 1 Least Square means (±SE) for agonistic contacts, displacements and licking behaviours of 6 high-aggressive and 5 low-aggressive Holstein cows, data measured on the day (d 0) and after the day (d +1 to +4) of regrouping.

Behavioural	Cow category	
	High-aggressive	Low-aggressive
Agonistic contacts (events)		
Initiated	26.0 ± 6.0	11.4 ± 6.0*
Received	25.8 ± 4.5	40.4 ± 4.8*
Displacement (events)		
Displaced others	13.7 ± 2.3	5.6 ± 2.5*
Displaced by others	12.3± 3.0	25.5 ± 3.4*
Social licking (events)		
Initiated	1.2 ± 0.4	1.2 ± 0.4
Received	1.0 ± 0.3	0.5± 0.3
Social licking duration (min)	3.6 ± 0.8	2.5 ± 0.9
Self-licking (events)	7.7 ± 1.1	8.2 ± 1.2

*Means in the same row differed significantly (P < 0.01)

3.4 Discussion

In order to define individual differences in aggression as a reflection of coping style, individuals would be expected to demonstrate consistent variation. The results of the current study indicate that consistent individual differences in aggression before and after social mixing, in both displacements and initiated aggression were present. Furthermore, the 2 indices (i.e. Displacement Index and Initiated Aggression Index) are closely correlated, suggesting that they represent a single trait. The measurements (before and after regrouping) involved almost completely different groups of pen-mates, indicating that the differences between cows are relatively consistent regardless of the group, and are therefore not merely a reflection of the dominance rank of the animals in a specific group.

Previous studies have also reported consistent and stable individual differences in aggression among mice and pigs (Koolhaas et al., 1999), and especially in larger animals such as cattle over years (Van Reenen et al., 2005). Van Reenen et al. (2002) also found a consistent individual variation in behavioural reactivity of cows to udder preparation between two stages of lactation.

Our results showed no differences between high-aggressive and low-aggressive cows either in the amount of social licking (number or duration) or in age at first calving. The present results of social licking contrasts with the study of Réale et al. (2010) who stated that high-aggressive (active coping) individuals are likely to have low sociability compared to low-aggressive (passive coping) individuals.

Previous studies have found that body weight, sex and age of animals are important factors in determining which animals win agonistic encounters (Jensen et al., 1996). For example, in pigs, body weight is often so strongly correlated with fighting ability that the largest animals in a group are likely to win a fight (Andersen et al., 2000). In other studies, however, aggressiveness characteristics of individuals were not affected by body weight of the intruder or the resident pig (Erhard and Mendl, 1997; D'Eath and Pickup, 2002). The present results also show that no association between body weight and Displacement Index of introduced cows, despite the heaviest introduced cow in the group having won all displacements after regrouping. These findings correspond with the findings of pigs by Erhard and Mendl (1997) and D'Eath and Pickup (2002). The consistent individual variation in aggression before and after regrouping, and the lack of relationship between aggression scores and body weight, seems to suggest that an individual difference in the propensity to be aggressive is independent of body weight.

Our results show no difference in milk production after regrouping between high-aggressive and low-aggressive cows. However, low-aggressive cows had greater projected 305-day ME milk production than high-aggressive cows. Hence, individual variation in aggressiveness may be associated with long-term effects as reflected by milk producing ability.

3.5 Conclusion

Results of the current study show that individual differences in aggressiveness among mid-lactation cows are fairly consistent characteristics before and after social mixing, and it correlates with the projected 305-day milk production, but not with body weight of individuals. This consistent individual variation in aggression before and after social mixing may reflect alternative coping styles of mid-lactation Holstein cows.

Chapter 4: General Discussions

4.1 Social stress

In modern dairy production, especially in North America, free-stall housing is typically associated with an increase in herd size and management practices that often results in increased social crowding and social mixing, which in turn causes social stress. Understanding the effects of management practices that perpetuate crowding and social mixing on the individual animals housed within these systems can provide useful insights with the aim of improving the welfare of gregarious animals (Rushen et al., 2008). The existence of individual differences in behaviour may also be important in understanding the complex social relations among animals living in a social group (Koolhaas et al., 1999).

The present work of mixing Holstein cows in pairs led to an increase in agonistic contacts and displacement behaviour for 3 d and 2 d, respectively, and showed that aggressiveness differences among mid-lactation cows is a consistent characteristic before and after regrouping. Collectively these results suggest this consistent individual variation in aggression may reflect alternative coping styles of individuals.

Previous work focusing on dairy cattle housed in free-stall housing has shown that a large number of physical agonistic interactions take place (DeVries and von Keyserlingk, 2005; Huzzey et al., 2006; Val-Laillet et al., 2009), and dairy cows are able to vary their feeding rate (Proudfoot et al., 2009) in response to increased stocking pressure. Social mixing also led to an increase of displacements and a decrease in time spent feeding (von Keyserlingk et al., 2008).

Although cows that were regrouped in the present study failed to show a decline in milk production on the day of, and subsequent days after regrouping, they did produce less milk than the resident cows in the new pen compared to the resident cows in their home pen. This finding may suggest that the degree of social stress arising following regrouping is greatest for those cows that are physically moved into a new pen but more work is needed to fully elucidate this finding. However, this result suggests that regrouping in pairs may mitigate, at least to some degree, the negative effects of regrouping on milk production.

Previous studies noted that regrouping is much more stressful for introduced cows compared to resident cows (Bøe and Færevik, 2003; Schirmann et al., 2011). In resident-intruder aggression test, intruder mice often trigger aggression at mixing but most often lose the fight to the resident mice (Hilakivi-Clarke and Lister, 1992), and are likely to sustain more social stress and injuries (Fokkema et al., 1995), especially for the aggressive intruder animals that lost the fights. The current agonistic results support this claim in that after mixing introduced focal cows received twice as many agonistic contacts as they initiated, and lost twice as many displacements as they won to resident cows, which may have contributed to the introduced focal cows producing less milk than resident cows.

Animals introduced singly into established social groups have been reported to engage in more agonistic contacts with the residents (Bøe and Færevik, 2003; Niesen et al., 2009), and integrate into new social group more easily (Gygax et al., 2009), and produce less milk (O'Connell et al., 2008) than animals introduced in pairs or a group of three. In contrast, Menke et al. (2000) found higher agonistic contact when heifers introduced in group of three into the social group compared to those heifers introduced singly.

In comparing the present study where cows were mixed in pairs with the previous study by von Keyserlingk et al. (2008) where cows were introduced individually, the average increase in the number of displacements received by introduced cows was similar in both studies. However, after regrouping, the decrease in milk production and social licking events of the present study did not differ from baseline. The presence of a familiar partner during mixing is worthy of consideration as a management practice that reduces social tension. This is supported by the result that after regrouping, the percentage of mutual social licking between pairs of regrouped cows is sharply increased, and these pairs were observed to stand together at the pen gate, follow one another in alleyways, and take neighbouring positions at the feed bunk especially on d 0 and d +1.

4.2 Linking social stressor and individual differences in aggression

Individual animals differ in many ways, including their response to a particular social stressor such as crowding and social mixing. Stress responses vary between species, breed, sex, age (body weight), as well as individual characteristics such as copying styles and social status within a group. Social status is more flexible than coping styles, indicating that social status of individuals can be changed depending on context, but its coping styles remain elemental to the animal (Lindberg (2001). Thus, the use of coping styles measures can be helpful to predict social stress responsiveness of individuals in advance (Schrader, 2002).

In many species, individual variation in aggression has shown a link to metabolic rate (Martins et al., 2011) and immunity (Koolhaas, 2008; Proudfoot et al., 2012). In sheep, low-aggressive males have been shown to have delayed reproduction and greater longevity (Réale et al., 2009), whereas high-aggressive ewes were associated with earlier age at first reproduction, and increased weaning success (Réale, et al., 2000). Furthermore, high-aggressive pigs also exhibited faster growth rates and produced piglets with heavier birth weight compared to low-aggressive pigs (Mendl et al., 1992). High-aggressive pigs were also shown to be at increased risk for injuries and slower group integration than low-aggressive pigs (Erhard et al., 1997). In farm animals, individual behavioural and neuroendocrine differences have also been linked to susceptibility to disease (Proudfoot et al., 2012).

In the present study, individual differences in aggressiveness showed an association with the 305-day milk production; namely, that low-aggressive cows produced more milk than high-aggressive cows, suggesting individual variation in aggression may be associated with long-term biological differences reflected in milk producing ability.

4.3 Limitations and future research

Studies of individual differences in many animal species have been related to the biological characteristics of the individual. Such differences may provide useful information, for example in dairy cattle, questions concerning why cows exposed to the same social environmental stressors show different stress responses, and why discrepancies exist in the degree of susceptibility to lameness and disease. Moreover, whether individual differences in aggressiveness can provide insights into why certain heifers show delayed reproduction and why some multiparous cows show reproduction (embryonic) failure remain. Other variables such as risk of survivability and longevity of cow may also be linked to individual variation in aggression and feeding behaviours are worthy of future research.

In other species, despite the fact that some work has shown that individual differences in aggression are linked to disease susceptibility, longevity and reproductive success or failure, such intraspecific variability in animal behaviour (e.g. aggression) is not yet fully elucidated. Much work remains to determine how knowledge regarding individual differences may impact management decisions and breeding practices on commercial dairy farms. Clearly, the aim is to prevent or alleviate adverse consequences, and to improve farm animal welfare in the dairy cattle industry.

My study provided a more in depth analyses of how social behaviour is impacted when cows are regrouped in pairs. Most interesting is that this is the first evidence suggesting a possible link between individual difference in aggression and milk producing ability of Holstein cows. This association between individual aggressiveness and milk production appears to be dependent, at least in part, on the temperament of the individual animal. This may be due to differences in neuroendocrine response to the lactogenic (prolactin and growth hormones) and oxytocin hormones but more work is required to fully understand these relationships.

The discussion presented in the current study, particularly Chapter 2, relied heavily on the work previously reported by von Keyserlingk et al. (2008). Ideally to fully elucidate the effects of regrouping cows in pairs it would have been beneficial to incorporate a treatment effect, for instance comparing the effects of regrouping a single cow or a pair. However, despite not being a controlled comparison I feel that there are some insights to be gained by comparing the studies, particularly because they were run in the same research facility. However, determining the effects of regrouping a pair of familiar cows verses two unfamiliar cows on social tension in a controlled study should also be investigated.

Another area of potential interest is to undertake additional research aimed at understanding the complex relationship that exists between animals housed together, and the role individual differences in aggression in mitigating social stress. Understanding the relationships between behavioural and physiological indicators of welfare and productivity, particularly with respect to the linkage between individual aggressiveness and susceptibility to diseases and lameness, reproductive success or failure and longevity of cows should also be explored. Lastly, more information is needed to fully understand the links between milk production and neuroendocrine physiological indicators of stress, such as secretion of milk synthesis and ejection hormones in response to social mixing cows.

References

Andersen, I. L., H. Andenaes, K. E. Bøe, P. Jensen, and M. Bakken. 2000. The effect of weight asymmetry and resource distribution on aggression in groups of unacquainted pigs. *Appl. Anim. Behav. Sci.* 68: 107-120.

Andersen, I. L., S. Roussel, E. Ropstad, B. O. Braastad, G. Steinheim, A. M. Janczak, G. M. Jørgensen, and K. E. Bøe. 2008. Social instability increases aggression in groups of dairy goats, but with minor consequences for goats' growth, kid production and development. *Appl. Anim. Behav. Sci*. 144: 132-148.

Benus, R. F., J. M. Koolhaas, and G. A. van Oortmerssen. 1987. Individual differences in behavioural reaction to a changing environment in mice and rats. *Behav*. 100: 105-122.

Biro, P. A., and J. A. Stamps. 2008. Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol*. 23: 361-368.

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

Boissy, A. 1995. Fear and fearfulness in animals. *Q. Rev. Biol*. 70: 165-191.

Boissy, A., G. Manteuffela, M. B. Jensen, R. O. Moe, B. Spruijt, L. J. Keeling, C. Winckler, B. Forkman, I. Dimitrov, J. Langbein, M. Bakken, I. Veissier, and A. Aubert. 2007. Assessment of positive emotions in animals to improve their welfare. *Physiol. Behav*. 92: 375-3977.

Bolhuis, J. E., W. G. P. Schouten, J. A. de Leeuwa, J. W. Schrama, and V. M. Wiegant. 2004. Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behav. Brain Res*. 152: 315-360.

Boon, A. K., D. Réale, and S. Boutin. 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecol. Lett.* 10: 1094-1104.

Brouns, F., and S. A. Edwards. 1994. Social rank and feeding behaviour of group-housed sows fed competitively or ad libitum. *Appl. Anim. Behav. Sci*. 39: 225-235.

Brouček, J., M. Uhrinčať, M. Šoch, and P. Kišac. 2008. Genetics of behaviour in cattle. *Slovak. J. Anim. Sci*. 41: 166-172.

Brouček, J., M. Uhrinčať, and A. Hanus. 2011. Maintenance and competitive behaviour study in dairy calves. *Slovak J. Anim. Sci.* 44: 28-33.

Camiloti, T. V., J. A. Fregonesi, M. A. G. von Keyserlingk, and D. M. Weary. 2012. Short communication: effects of bedding quality on the lying behaviour of dairy calves. *J. Dairy Sci*. 95: 3380-3383.

Cannon, W. B. 1929. Bodily Changes in Pain, Hunger, Fear and Rage, 2nd ed. Appleton, New York.

Carere, C., D. Caramaschi, and T. W. Fawcett. 2010. Covariation between personalities and individual differences in coping with stress: Converging evidence and hypotheses. *Current Zool*. 56: 728-740.

CCAC, 2009. CCAC guidelines on the care and use of farm animals in research, teaching and testing. Canadain Council on Animal Care (CCAC), ottawa.

CDIC. 2011. Dairy facts and figures. Canadian Dairy Information Centre (CDIC). Accessed February 7, 2012 from

http://www.dairyinfo.gc.ca/index_e.php?s1=dff-fcil

Christensen, J. W., E. Søndergaard, K. Thodberg, and U. Halekon. 2011. Effects of repeated regrouping on horse behaviour and injuries. *Appl. Anim. Behav. Sci*. 133: 199-206.

Coppens, C. M., S. F. deBoer, and J. M. Koolhaas. 2010. Coping styles and behavioural flexibility towards underlying mechanisms. *Phil. Trans. R. Soc. B* 365: 4021-4028.

Costantini, D., C. Carere, D. Caramaschi, and J. M. Koolhaas. 2008. Aggressive and non-aggressive personalities differ in oxidative status in selected lines of mice. *Biol. Lett*. 4: 119-122.

Costantini, D., C. Ferrari, C. Pasquaretta, E. Cavallone, C. Carere, A. von Hardenber, and D. Réale. 2012. Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, *Marmota marmota*. *J. Exp. Biol*. 215: 374-383.

D'Eath, R. B. 2002. Individual aggressiveness measured in resident-intruder test predict the persistence of aggressive behaviour and weight gain of young pigs after mixing. *Appl. Anim. Behav. Sci*. 77: 267-283.

D'Eath, R. B., and C. C. Burn. 2002. Individual difference in behaviour: a test of coping style does not predict resident-intruder aggressiveness in pigs. *Behav*. 93: 1175-1194.

D'Eath, R. B., and H. E. Pickup. 2002**.** Behaviour of young growing pigs in a resident-intruder test designed to measure aggressiveness. *Aggr. Behav*. 28: 401-415.

DeVries, T. J., M. A .G. von Keyserlingk, and K.A. Beauchemin. 2003. Short Communication: Diurnal feeding pattern of lactating dairy cows. *J. Dairy Sci*. 56: 4079-4082

DeVries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behaviour of free-stall housed lactating dairy cows. *J. Dairy Sci*. 87: 1432-1438.

DeVries, T. J., and M. A. G. von Keyserlingk. 2005. Time of feed delivery affects the feeding and lying patterns of dairy cows. J. Dairy Sci. 88: 625-631.

DeVries, T. J., M. A. G. von Keyserlingk and K. A. Beauchemin. 2005. Frequency of feed delivery affects the behaviour of lactating dairy cows. *J. Dairy Sci*. 88: 3553-3562.

DeVries, T. J., and M. A. G. von Keyserlingk. 2006. Feed stalls affect the social and feeding behaviour of lactating dairy cows. *J. Dairy Sci*. 89: 3522-3531.

Dindo, M., A. Whiten and F. B. M. DeWaal. 2009. Social facilitation of exploratory foraging behaviour in Capuchin monkeys (*Cebus apella*). *Amer. J. Primatol*. 71: 419-426.

Driscoll, P., J. R. Martin, P. Kugler, and K. Baettig. 1983. Environmental and genetic effects on food-deprivation induced stomach lesions in male rats. *Physiol. Behav.* 31: 225-228.

Drissler, M., M. Gaworski, C. B. Tucker, and D. M. Weary. 2005. Free stall maintenance: effects on lying behaviour of dairy cattle. *J. Dairy Sci*. 88: 2381-2387.

Engel, G. L., and A. H. Schmale. 1972. Conservation withdrawal: A primary regulatory process for organic homeostasis. In: Porter, R., Knight, J. ed. Physiology, Emotions and Psychosomatic Illness. Elsevier Science Publishers, New York, pp. 57-76.

Erhard, H. W., and M. Mendl. 1997. Measuring aggressiveness in growing pigs in a resident-intruder situation. *Appl. Anim. Behav. Sci.* 54: 123-136.

Erhard, H. W., M. Mendl, and D. D. Ashley. 1997. Individual aggressiveness of pigs can be measured and used to reduce aggression after mixing. *Appl. Anim. Behav. Sci*. 54: 137–151.

Fernández, M. A., L. Alvarez, and L. Zarco. 2007. Regrouping in lactating goats increases aggression and decreases milk production. *Small Ruminant Res*. 70: 228-232.

Ferrante,V., E. Canali, S. Mattiello, M. Verga, P. Sacerdote, B. Manfredi, and A. E. Panerai. 1998. Preliminary study on the effect of size of individual stall on the behavioural and immune reactions of dairy calves. *J. Anim. Feed Sci*. 7: 29-36.

Fokkema, D. S., J. M. Koolhaas, and J. Van Der Gugten. 1995. Individual characteristics of behaviour, blood pressure, and adrenal hormones in colon rats. *Physiol. Behav*. 57: 857-862.

Forkman, B., I. L. Furuhaug, and P. Jensen. 1995. Personality, coping patterns, and aggression in piglets. *Appl. Anim. Behav. Sci*. 45: 31-42.

Fraser, D., 2008. Understanding Animal Welfare: The Science in its Cultural Context. Wiley-Blackwell, Oxford.

Fraser, A. F., and D. M. Broom. 1990. Farm Animal Behaviour and Welfare, 3rd ed. Baillière Tindall, London.

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

Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007a. Overstocking reduces lying time in dairy cows. *J. Dairy Sci*. 90: 3349-3354.

Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007b. Effects of bedding on lying behaviour of dairy cows. *J. Dairy Sci.* 90: 5468-5472.

Galindo, F., and D. M. Broom. 2000. The relationship between social behaviour of dairy cows and the occurrence of lameness in three herds. *Res. Vet. Sci*. 69: 75-79.

Grant, R. J., and J. L. Albright. 2001. Effect of animal grouping on feeding behaviour and intake of dairy cattle. *J. Dairy Sci.* 84: 156-163.

Geverink, N. A., W. G. P. Schouten, G. Gort, and V. M. Wiegant. 2003. Individual differences in behaviour, physiology and pathology in breeding gilts housed in groups or stalls. *Appl. Anim. Behav. Sci.* 81: 29-41.

González, M., A. K. Yobuta, and F. Galindo. 2003. Behaviour and adrenal activity of first parturition and multiparous cows under a competitive situation. *Appl. Anim. Behav. Sci*. 83: 259-266.

Gupta, S., B. Earley, M. Nolan, E. Formentin, and M. A. Crowe. 2008. Effect of repeated regrouping and relocation on behaviour of steers. *Appl. Anim. Behav. Sci.* 110: 229-243.

Gygax, L., G. Neisen, and B. Wechsler. 2009. Differences between single and paired heifers in residency in functional areas, length of travel path, and area used throughout days 1–6 after integration into a free stall dairy herd. *Appl. Anim. Behav. Sci*. 120: 49-55.

Haller, J., M. Toth, J. Halasz, and S. F. de Boer. 2006. Patterns of violent aggression-induced brain *c-fos* expression in male mice selected for aggressiveness. *Physiol. Behav.* 88: 173-182.

Hansen, L. T., and H. Berthelsen. 2000.The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*). *Appl. Anim. Behav. Sci*. 68: 163-178.

Hasegawa, N., A. Nishiwaki, K. Sugawara, and I. Ito. 1997. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behaviour and adrenocorical resonse. *Appl. Anim. Behav*. Sci. 51: 15-27.

Haye, S. M., and H. W. Gonyou. 2006. Behavioural uniformity or diversity? Effects on behaviour and performance following regrouping in pigs. *Appl. Anim. Behav. Sci*. 98: 28-44.

Henry, J. P., and P. M. Stephens. 1977. Stress, Health, and the Social Environment: Sociobiological Approach to Medicine. Springer, New York.

Herborn, K., L. Alexander, and K. E. Arnold. 2011. Colour cues or spatial cues? Context-dependent preferences in the European greenfinch (*Carduelis chloris*). *Anim. Cogn*. 14: 269-277.

Herskin, M. S., A. M. Kristensen, and L. Munksgaard. 2004. Behavioural responses of dairy cows toward novel stimuli presented in the home environment. *Appl. Anim. Behav. Sci*. 89: 27-40.

Hessing, M. J. C., A. M. Hagelsø, J. A. M. van Beek, P. R. Wiepkema, W. G. P. Schouten, and R. Krukow. 1993. Individual behavioural characteristics in pigs. *Appl. Anim. Behav. Sci.* 37: 285-295.

Hessing, M. J. C., A. M. Hagelsø, W. G. Schouten, P. R. Wiepkema, and J. A. M. van Beek. 1994. Individual behavioural and physiological strategies in pigs. *Physiol. Behav*. 55: 39-46.

Hilakivi-Clarke, L. A., and R. G. Lister. 1992. The role of body weight in resident-intruder aggression. *Aggressive . Behav*. 18: 281.287.

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, W. A. Falls, and R. J. Grant. 2009. Effect of stocking density on the short-term behavioural response of dairy cows. *Appl. Anim. Behav. Sci*. 117: 144-149.

Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behaviour of dairy cattle. *J. Dairy Sci*. 89: 126-133.

Jensen, P. 1995. Individual variation in the behaviour of pigs noise or functional coping strategies? *Appl. Anim. Behav. Sci.* 44: 245-255.

Jensen, P., B. Forkman, J. Yngvesson, and I. L. Furuhaug. 1996. Assessment in pig conflicts. Page 46. In: *Proceedings of 30th International Congress of the ISAE*. Guelph, Ontario, pp 46.

Kavelaars, A., C. J. Heijnen, R. Tennekes, J. E. Bruggink, and J. M. Koolhaas. 1999. Individual behavioural characteristics of wild-type rats predict susceptibility to experimental autoimmune encephalomyelitis. *Brain Behav. Immun.* 13: 279-286.

Kemble, E. D. 1993. Resident-intruder paradigms for the study of rodent aggression. In: Conn, P. M. ed. Methods in Neurosciences: Paradigms for the Study of Behaviour. Academic Press, New York, vol 14. pp. 138–150.

Kerr, S. G. C., and D. G. M. Wood-Gush. 1987. Comparison of the early behaviour of intensively and extensively reared calves. *Anim. Prod*. 45: 181-190.

Kondo, S., and J. F. Hurnik. 1990. Stabilization of social hierarchy in dairy cows. *Appl. Anim. Behav. Sci*. 27: 287-297.

Koolhaas, J. M., S. M. Korte, S. F. De Boer, B. J. Van der Vegt, C. G. Van Reenen, H. Hopster, I. C. De Jong, M. A. Ruis, and H. J. Blokhuis. 1999. Coping styles in animals: Current status in behaviour and stress physiology. *Neurosci. Biobehav. Rev*. 23: 925- 935.

Koolhaas, J. M. 2008. Coping style and immunity in animals: Making sense of individual variation. *Brain Behav. Immun*. 22: 662-667.

Koolhaas, J. M., S. F. De Boer, C. M. Coppens, and B. Buwalda. 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Front. Neuroendocrin*. 31: 307-321

Korte, S. M., W. Ruesink, and H. J. Blokhuis. 1999. Heart rate variability during manual restraint in chicks from high- and low-feather pecking lines of laying hens. *Physiol. Behav*. 65: 649-652.

Lindberg, C. 2001. Group life. In: Keeling, L. J., H. W. Gonyou (eds), Social Behaviour in Farm Animals. CABI, p.406.

Laister, S., B. Stockinger, A. M. Regner, K. Senger, U. Knierim, and C. Winckler. 2011. Social licking in dairy cattle effects on heart rate in performers and receivers. *Appl. Anim. Behav. Sci*. 130: 81-90.

Manning, A., and M. S. Dawkins. 1992. An Introduction to Animal Behaviour, 6th ed. Cambridge University Press, Cambridge.

Martins, C. I. M., M. F. Castanheira, S. Engrola, B. Costas, and L. E. C Conceićão. 2011. Individual differences in metabolism predict coping styles in fish. *Appl. Anim. Behav. Sci*. 130: 135-143.

Mendl, M., A. J. Zanella, and D. M. Broom. 1992. Physiological and reproductive correlates of behavioural strategies in female domestic pigs. *Anim. Behav*. 44: 1107-l 121.

Mendl, M., and J. M. Deag. 1995. How useful are the concepts of alternative strategy and coping strategy in applied studies of social behaviour? *Appl. Anim. Behav. Sci*. 44: 119-137.

Menke, C., S. Waiblinger, and D. W. Fölsch. 2000. Die Bedeutung von Managementmassnahmen im Lausfstall für das Sozialverhalten von Milchkühen (in German with English summary). Deutsche tierärztliche Wochenschrift. 107: 262-268.

Miranda-de la Lama, G. C., M. Villarroel, and G. A. María. 2012. Behavioural and physiological profiles following exposure to novel environment and social mixing in lambs. Small Ruminant Res. 103: 158-163.

Munksgaard, L., and H. B. Simonsen. 1996. Behavioural and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. *J. Anim. Sci*. 74: 769-778.

Nakanishi, Y., T. Kawamura, T. Goto, and R. Umetso. 1993. Comparative aspects of behavioural activities of beef cows before and after introducing a stranger at night. *J. Fac. Agricult. Kyushu. Univ*. 37: 227-238.

Napolitano, F., U. Knierim, F. Grasso, and G. De Rosa. 2007 Positive indicators of cattle welfare and their applicability to on-farm protocols**.** *Ital. J. Anim. Sci.* 8: 355-365.

Neisen, G., B. Wechsler, and L. Gygax. 2009. Effects of the introduction of single heifers or pairs of heifers into dairy-cow herds on the temporal and spatial associations of heifers and cows. *Appl. Anim. Behav. Sci.* 119: 127-136.

O'Connell, N. E., H. C. F. Wicks, A. F. Carson, and M. A. McCoy. 2008. Influence of post-calving regrouping strategy on welfare and performance parameters in dairy heifers. *Appl. Anim. Behav. Sci*. 114: 319-329.

OIE, 2011. Terrestrial Animal Health Code, 20th ed. World Organisation for Animal Health (OIE), Paris, pp. 289-290.

Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. *J. Dairy Sci*. 82: 69-79.

Patison, K. P., D. L. Swain, G. J. Bishop-Hurley, G. Robins, P. Pattison, and D. J. Reid. 2010. Changes in temporal and spatial associations between pairs of cattle during the process of familiarisation. *Appl. Anim. Behav. Sci*. 128: 10-17.

Patt, A. Gygax, B. Wechsler, E. Hillmann, R. Palme, and N. M. Keil. 2012. The introduction of individual goats into small established groups has serious negative effects on the introduced goat but not on resident goats. *Appl. Anim. Behav. Sci*. 138: 47-59

Phillips, C. J. C., and M. I. Rind. 2001. The effects on production and behaviour of mixing uniparous and multiparous cows. *J. Dairy Sci*. 84: 2424-2429.

Proudfoot, K. L., D. M. Veira, D. M. Weary, and M. A. G von Keyserlingk. 2009. Competition at the feed bunk changes the feeding, standing and social behaviour of transition dairy cows. *J. Dairy Sci*. 92: 3116-3123.

Proudfoot, K. L., D. M. Weary, and M. A. G. von Keyserlingk. 2012. Linking the social environment to illness in farm animals. *Appl. Anim. Behav. Sci.* 138: 203-215.

Rammal, H., J. Bouayed, and R. Soulimanl. 2010. A direct relationship between aggressive behaviour in the resident-intruder test and cell oxidative status in adult male mice. *Eur. J. Pharmacol*. 627: 173-176.

Réale, D., B. Y. Gallant, M. Leblanc, and M. Festa-Bianchet. 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Anim. Behav*. 60: 589-597.

Réale, D, S. M. Reader, D. Sol, P. T. McDougal, and N. J. Dingemanse. 2007. Integrating animal temperament within ecology and evolution. *Biol. Rev*. 82: 291-318.

Réale, D., J. Martin, D. W. Coltman, J. Poissant, and M. Festa-Bianchet. 2009. Male personality, life-history strategies and reproductive success in a promiscuous mammal. *J. Evol. Biol.* 22: 1599-1607.

Réale, D., D. Garant, M. M. Humphries, P. Bergeron, V. Careau, and P. O. Montiglio. 2010. Personality and the emergency of the pace-of-life syndrome concept at the population level. *Phil. Trans. R. Soc. B* 365: 4051-4063.

Reich, L. J., D. M. Weary, D. M. Veira, and M. A. G. von Keyserlingk. 2010. Effect of sawdust bedding dry matter on lying behaviour of dairy cows: a dose-dependent response. *J. Dairy Sci.* 93: 1561-1565.

Ruis, M. A. W., J. H. A. Te Brake, J. A. Van de Burgwal, I. C. De Jong, H. J. Blokhuis, and J. M. Koolhaas. 2000. Personalities in female domesticated pigs: behavioural and physiological indications. *Appl. Anim. Behav. Sci*. 66: 31-47.

Rushen, J., A. M. de Passillé, M. A. G. von Keyserlingk, and D. M. Weary. 2008. The Welfare of Cattle. Springer, Dordrecht.

Salmon, A. B., A. Richardson, V. I. Pérez. 2010. Update on the oxidative stress theory of aging: Does oxidative stress play a role in aging or healthy aging. *Free Radic. Biol. Med*. 48: 642-655.

Samarakone T. S., and Gonyou H. W. (2008) Productivity and aggression at grouping of grower finisher pigs in large groups. *Can. J. Anim. Sci.* 88: 9-17.

SAS. 2011. Statistical Analysis System (SAS) options, Version 9.3, 2nd ed. SAS Inst., Inc., Cary, NC.

Sato, S., and K. Tarumizu. 1993. Heart rates before, during and after allo-grooming in cattle (*Bos taurus*). *J. Ethol*. 11: 149-150.

Sato, S., K. Tarumizu, and K. Hatae. 1993. The influence of social factors on social licking in cows. *Appl. Anim. Behav. Sci*. 38: 235-244.

Schrader, L. 2002. Consistency of individual behavioural characteristics of dairy cows in their home pen. *Appl. Anim. Behav. Sci.* 77: 255-266.

Schirmann, K., N. Chapinal, D. M. Weary, W. Heuwieser, and M. A. G. von Keyserlingk. 2011. Short-term effects of regrouping on behaviour of prepartum dairy cows. *J. Dairy Sci.* 94: 2312-2319.

Sevi, A., L. Taibi, M. Albenizio, A. Muscio, S. Dell-Aquila, and F. Napolitano. 2001. Behavioural, adrenal, immune and productive responses of lactating ewes to regrouping and relocation. *J. Anim. Sci*. 79: 1457-1465.

Sih, A., A. M. Bell, and J. C. Johnson. 2004. Behavioural syndromes: An ecological and evolutionary overview. *Trends Ecol. Evol*. 19: 372-378.

Smith, B. R., and D. L. Blumstein. 2008. Fitness consequences of personality: A meta-analysis. *Behav. Ecol*. 19: 448-455.

Stookey, J. M. 1994. Is Intensive Dairy Production Compatible With Animal Welfare? Pages 209-219 In: *Advances in Dairy Technology Vol. 6. Proceedings of the Western Dairy Canadian Dairy Seminar*.

Takeda, K., S. Sato, and K. Sugawara. 2000. The number of farm mates influences social and maintenance behaviours of Japanese black cows in a communal pasture. *Appl. Anim. Behav. Sci*. 67: 181-192.

Takeda, K., S. Sato, and K. Sugawara. 2003. Familiarity and group size affect emotional stress in Japanese black heifers. *Appl. Anim. Behav. Sci*. 82: 1-11.

Tucker, C. B., and D. M. Weary. 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort. *J. Dairy Sci.* 87: 2889-2895.

Tucker, C. B., D. M. Weary, M. A. G. von Keyserlingk, and K. A. Beauchemin. 2009. Cow comfort in tie-stalls: Increased depth of shavings or straw bedding increases lying time. *J. Dairy Sci*. 92: 2684-2690.

Val-Laillet, D., V. Guesdon, M. A. G. von Keyserlingk, A. M. de Passillé, and J. Rushen. 2009. Allogrooming in cattle: relationships between social preferences, feeding displacements and social dominance. *Appl. Anim. Behav*. 116: 141-149.

Van Reenen, C. G., J. T. N Van der Werf, R. M. Bruckmaier, H. Hopster, B. Engel, J. P. T. M. Noordhuizen, and H. J. Blokhuis. 2002. Individual differences in behavioural and physiological responsiveness of primiparous dairy cows to machine milking. *J. Dairy Sci*. 85: 2551-2561.

Van Reenen, C. G., N. E. O'Connaell, J. T. Van der Werf, S. M. Korte, H. Hopster, R. B. Jones, and H. J. Blokhuis. 2005. Responses of calves to acute stress: individual consistency and relations between behavioural and physiological measures. *Physiol. Behav*. 85: 557-570.

Verbeek, M. E. M., P. de Goede, P. J. Drent, and P. R. Wiepkema. 1999. Individual behavioural characteristics and dominance in aviary groups of great tits. *Behav*. 136: 23-48.

von Keyserlingk, M. A. G., D. Olenick, and D. M. Weary. 2008. Acute behavioural effects of regrouping dairy cows. *J. Dairy Sci*. 91: 1011-1016.

Wechsler, B. 1995. Coping and coping style: a behavioural view. *Appl. Anim. Behav. Sci.* 43: 123-134.