THE EFFECTS OF REGROUPING AND STOCKING DENISTY ON SOCIAL BEHAVIOUR, LYING BEHAVIOUR AND LOCOMOTOR ACTIVITY OF MID AND LATE LACTATION DAIRY COWS

by

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ABSTRACT

In free-stall systems cows are frequently moved among pens and regrouped. This practice involves individuals being mixed with unfamiliar cows, and stocking density often varies from before to after regrouping. Two separate lines of evidence suggest that regrouping and changing densities have negative impacts on cows welfare; but no study to date has assessed the combined effects. The aim of this study was to test the effect of changes in stocking density at the time of regrouping on the competition, feeding and lying behaviours and locomotor activity of dairy cows. By manipulating group size (6 vs. 12 cows) and pen size (12 vs. 24 stalls) three different stocking densities were created (25 %, 50 % and 100 %). Four groups of Holstein cows were regrouped weekly for 4 weeks and the stocking density changed from before to after each regrouping. The change in density varied from a decrease by a factor of 4 (100% to 25%), a decrease by a factor of 2 (100% to 50% or 50% to 25%), no change (50% to 50%), an increase by a factor of 2 (25% to 50% or 50% to 100%) and an increase by a factor of 4 (25% to 100%). Displacement at the feeding area, feeding time, lying time and the number of steps were scored. The daily means for each group were used to calculate the difference in responses from one day before to one day after each regrouping. Competition at the feed bunk changed after regrouping. The nature of the change was dependent upon the change in stocking density; when density decreased the number of displacements decreased. Changes in lying behavior and locomotor activity after regrouping also varied with changes in stocking density; when stocking density decreased lying time increased and number of steps increased. In conclusion, results of this experiment show that the change in competition behaviour from one day before to one day after regrouping varies

with the change in stocking density at regrouping; and this change in competition results in changes in lying time and locomotor activity of cows.

PREFACE

The current study was designed through the collaborative work of Aniseh Talebi and Drs. Dan Weary, Marina von Keyserlingk and Evjenij Telezhenko. Aniseh Talebi was in charge of fulfillment of the project. She conducted the experiment, collected data, analyzed data, reviewed literature, interpreted results and wrote the manuscript under the supervision of Drs Dan Weary and Marina von Keyserlingk. Dr. Evjenij Telezhenko helped in running the first replication and collection of data in October 2009.

A version of Chapter 2 is going to be submitted for publication: Talebi, A., von Keyserlingk, M.A.G., Telezhenko, E., Weary, D.M., 2011. Increased stocking density compounds the negative effects of regrouping on social behaviour, lying behaviour and locomotor activity of dairy cows.

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DEDICATION

I dedicate this thesis to the animals in Iran from domestic to wild, from the Caspian sea in the North to the Persian Golf in the South, from the Lout desert in the East to the Zagros mountains in the West, hoping for a day that science-based solutions will be adopted in Iran, helping animals experience a better life.

CHAPTER 1: GENERAL INTRODUCTION

1.1. DAIRY PRODUCTION AND COW WELFARE

Animal welfare is a growing area of research greatly influenced by social and ethical concerns (Fraser, 2008). Three main areas of concern have been identified: 1) the animal's biological functioning, 2) the animal's affective state and, 3) the animal's ability to live a natural life (Fraser et al., 1997). These three concerns often overlap. For example, in a pen that is overstocked, a lower ranked cow is often required to compete for resources with higher ranked cows. Moreover, in confinement conditions, she is forced to remain in this competitive situation and is unable to leave the group as wild cattle would under natural conditions (natural behaviour). The cow may fear the higher ranked animals and thus withdraw from competition for feed and thereby experience hunger (affective state). These changes may result in a physiological stress response (e.g. release of corticosteroids) that can in turn compromise her ability to cope with disease (biological functioning). The "Five freedoms" is another well-known approach toward animal welfare. These are: 1) freedom from hunger and thirst, 2) freedom from pain, injury and disease, 3) freedom from discomfort, 4) freedom from fear and distress, and 5) freedom to express natural behaviour (Webster, 1994). Although, the five freedoms are broad goals toward which scientists and researchers should move they are not ways of measuring animal welfare (Rushen et al., 2008).

Two main criteria have been used to assess animal welfare: 1) environment-based criteria (input-based) such as housing, management and feeding, and 2) animal-based criteria (outcome-based) such as behaviour, physiology and production (Mench, 2003). The advantage of environment-based criteria is that they can be used to set standards for housing, management and feeding systems and ideally help to prevent welfare problems that result from mismanagement and inappropriate facilities (Rushen et al., 2008). The advantage of animal-based criteria is that they give researchers the opportunity to measure the state of animals (Rushen et al., 2008). Although many researchers have tried to combine these two criteria to study animal welfare, we lack information on how particular environment-based criteria such as pen size and group size affect animal-based criteria such as feeding and competitive behaviour.

In the last few decades, the effects of dairy production systems on welfare have become topics of increasing interest for researchers. Concerns about the welfare of cattle have focused on housing and techniques used in intensive farming systems, in addition to more traditional concerns around health and nutrition. One specific question is how a particular facility characteristic and management practice influences cows' welfare, and how these can be improved to provide a more comfortable life for these animals.

One of the main characteristics of intensive dairy farms is that they provide limited indoor space for the animals. Tie-stall and free-stall systems are two common types of intensive housing for dairy cows. In tie-stall systems an individual cow is tethered in its stall where she is provided with space that is used both for standing and lying. She is not able to turn around and as a consequence of the tethering may have limited ability to groom herself. Food and water are provided individually and cows are often milked within the stall. In tie-stall systems animals are provided with a guaranteed amount of food and water and lying area with no competition, but this housing system can limit the cow's movement and decrease her physical interaction with other cows (Rushen, et al., 2008). In free-stall systems, the feeding and lying areas are separate and cows can move freely between them. Within the pen, cows have free access to food and water and are usually milked in a milking parlour unit, requiring them to leave the pen at least twice daily. Free-stall systems provide the opportunity for the cows to have social contact with other cows and some exercise (Bøe and Færevik, 2003) but depending on the stocking density, cows are often required to compete for access to food, water and lying spaces.

Free-stall systems are also often managed in ways that may negatively impact cow welfare. For instance, in order to feed rations based on stage of lactation, cows are frequently moved among pens and regrouped. This practice results in individuals being mixed with unfamiliar cows; furthermore, the numbers of animals in each pen varies. It has been shown that such mixing and changing of animal numbers in pens can increase physical competition, restrict access to resources (e.g feeding areas and lying stalls) and compromise animal welfare (Zeeb et al., 1988; Miller and Woodgush, 1991; Olofsson, 1999).

1.2. REGROUPING

Cattle establish groups under natural conditions. Formation of cooperative relationships and competitive interactions are the main consequences of group life. Some of the benefits of being a member of a group for cattle are that the cows are able to communicate information about the environment with other members of the group (e.g. food, predators), protect themselves from predators, spend more time foraging and save energy in cold weather by huddling together (Keeling and Gonyou, 2001).

In intensive cattle production, groups are formed based on the management requirements of the producer. Bøe and Færevik (2003) have defined *grouping* as "the formation of a group of animals by natural means (e.g., herd formation as a result of social attraction) or by human action (e.g., allocation of a number of animals to a given pen or grouping of dairy cows according to milking performance)". In free-stall systems, cows that are kept within a pen have the advantage of interacting with other cows and establishing affiliative relationships with other members of the group (Keeling and Gonyou, 2001). However, there are some costs associated with group life. For example, in group life there is a higher chance of contact-transmitted infectious diseases (Fraser and Broom, 2007), and cows housed in a pen with limited feeders and stalls relative to the numbers of cows must compete for these resources (Val-Laillet et al., 2008).

In free-stall systems a cow can be grouped with other cows 4 to 5 times during one lactation (Grant and Albright, 2001). Grouping animals repeatedly is called *regrouping* (also referred to as comingling and mixing; Bøe and Færevik, 2003). Animals are regrouped to form homogenous pens in terms of age, body condition, stage of lactation, milk production, health and reproduction status (Grant and Albright, 2001; Bøe and Færevik, 2003). Having homogenous groups of animals facilitates farm management; for example, it will allow rations to more adequately match the nutritional requirements of individual animals in a group pen (Grant and Albright, 2001).

When a cow joins a group of cows that have an established social order, a new order must be established (Bøe and Færevik, 2003). Social order refers to how individuals rank relative to other animals in the group (Keeling and Gonyou, 2001). Before each regrouping, interactions among group members tend to be non-physical, but after grouping this shifts towards more physical interactions with other members of the new group (Bøe and Færevik, 2003). Such interactions persist until a stable social order is re-established (Lamb, 1975; Kondo and Hurnik, 1990). Kondo and Hurnik (1990) have defined *social stabilization* as "when non-physical agonistic interactions among group members predominate and the ratio of physical to non-physical interaction remains comparatively stable". Changes in social behaviour due to regrouping typically return to normal in approximately 3 days to 2 weeks after regrouping (e.g. Kondo et al., 1984; Hasegawa et al., 1997; von Keyserlingk et al., 2008).It has been suggested that establishment of a new social order requires approximately one week for dairy cattle (Grant and Albright, 2001; Bøe and Færevik, 2003). The majority of research investigating aspects of social behaviour provide at least one week for the animals to stabilize after each regrouping (Nordlund et al., 2006).

Although competition can always occur among group housed animals (Estevez et al., 2007), repeated grouping of animals increases competitive interactions and interferes with the daily time budget of cows resulting in decreased feeding time and lying time (e.g. von Keyserlingk et al., 2008; Hasegawa, et al., 1997). Although all members of the new group may be affected by the negative consequences of regrouping, it is believed that lower ranked animals are particularly influenced (Bøe and Færevik, 2003). Regrouping may have profound effects on cow behaviour and welfare (Bøe and Færevik, 2003), and farmers require science-based solutions for reducing competitive interactions when forming new groups.

It has been shown that frequent groupings disturb social behaviour in several species. For example, after being grouped with unfamiliar animals, pigs compete more aggressively with other group members to gain access to limited resources (e.g. McGlone and Curtis, 1985; Stookey and Gonyou; 1994). Regrouping disturbs social behaviour and increases aggressive behaviour among laying hens (e.g. Abeyesinghe et al., 2009).

Research on regrouping in dairy cows is minimal. Many of the previous studies on regrouping had not enough numbers of replications (e.g. Hasegawa et al., 1997; Philips and Rind, 2001); other studies are based on limited sample sizes (e.g., Brakel and Leis, 1976; von Keyserlingk et al., 2008); although small groups can be more easily manipulated, the responses may differ from real situations where larger numbers of cows are grouped together.

It is therefore important for new research to focus on the effects of regrouping in larger groups of cows.

1.3. STOCKING DENSITY

Stocking density is defined as the “number of animals per unit of space” and the inverse of stocking density is *space allowance* that is “area of space per animal” (Estevez et al., 2007). As is implied in the definition, group size and pen size are the two factors affecting the stocking density. Stocking density is usually described as the numbers of animals per resource e.g. stall or the feeding area available for each cow. The result of adding animals to a given area or a reduction of the area available to a given numbers of animals is an increase in stocking density (Grant and Albright, 2001; Rushen, et al., 2008). Higher stocking densities may negatively impact the welfare of animals. For example, in highly stocked pens poor air quality or accumulation of manure on the floor may result in health problems such as pneumonia and laminitis (Bowell et al., 2003; Fraser and Broom, 2007).

The negative impact of high stocking densities also has been shown in other species. For example, in laying hens, increased densities can lead to higher mortality, increased feather damage and cannibalism (Simonsen et al., 1980; Adams and Craig, 1984). It has also been shown that sheep housed indoors engage in more competitive behaviour when they have restricted access to feeding troughs due to increased stocking density (Dove, et al., 1974).

Behavioural measures are considered to be important indicators of the cow’s welfare in response to changes in housing and management such as changes in stocking density, group size and pen size (Nordlund et al., 2006; Rushen, et al., 2008). For example, Proudfoot et al. (2009) showed that transition cows (i.e. from 3 weeks before calving to 3 weeks after calving) reduce their feeding time and increase their standing time when overstocked at the feeder. In pigs, stress induced by reducing pen size or increasing group size can decrease daily feed intake and appetite in these animals (Walker, 1991; Gonyou and Stricklin, 1998).

Dairy cattle may experience a range of stocking densities over their life. For example, calves are usually housed individually (i.e. one calf per crate), but after weaning they are put in pens with other heifers forming groups of larger sizes and different densities. At the beginning of their first lactation, heifers are typically grouped with more mature cows forming even larger groups. Research on stocking density has focused on cows in very early and mid stages of lactation, and little research has focused on the effect of stocking density later in lactation (Nordlund et al., 2006). Of the studies available, most show negative effects of increased stocking density on the cow behaviour and welfare. For instance, Fregonesi et al. (2007) found that changing stocking densities from 100% to 150% caused cows to reduce lying time from 12.9 h/d to 11.2 h/d. Similarly, Hill et al. (2009) found that when stocking density increased from 100% to 140%, lying time decreased by over an hour per day.

The majority of previous studies have focused on the effects of overstocking (i.e. less than one stall or less than 60 cm feeding area per cow (e.g. Fregonesi et al., 2007; Hill et al., 2009). On dairy farms, regrouping is often associated with changes in stocking density. For example, a cow may be moved from a pen with a lower stocking density to a pen with a higher stocking density. In addition, the majority of empirical studies undertaken on stocking density are done by either blocking off stalls or feeding positions.

1.3.1. Group size

Numbers of cows in a pen (i.e. group size) varies widely depending on the management requirements, herd size and farm facilities (Grant and Albright, 2001). One of the costs of being in a large group of animals is that there is more opportunity for competitive encounters over resources (Keeling and Gonyou, 2001; Fraser and Broom, 2007). Behavioural responses to changes in group size have been examined in some farm animal species. For instance, research in laying hens showed that aggressive interactions increase when group size increases (Oden et al., 2000; Estevez et al. 2007). Bøe and Færevik (2003) speculated that a relationship existed between level of aggression and group size in dairy cattle; increasing the number of cows per unit of space will increase the aggressive encounters. Few studies have tested the effect of number of cows introduced to the group on competitive behaviour. Kondo et al. (1989) showed a strong correlation between group size and competitive behaviour in heifers and adult cows but not in calves. To the author's knowledge no study to date has tested how group size influences behaviour of dairy cows after regrouping and changing density.

1.3.2. Pen size

The minimum space around the body that animals normally maintain between one another is defined as *individual distance* (Wilson, 2000). For example, horses try to maintain relatively large individual distances from the higher ranked animals when feeding (Fraser and Broom, 2007). Increasing numbers of animals per space (i.e. increasing stocking density) may make it difficult for the animals to keep their desired distance from other animals. It has been suggested that decreased individual distance may impact some individuals more than others. For instance, group order is often maintained by less aggressive animals performing subtle avoidance behaviours; however, limited space allowance makes it difficult for lower ranked animals to avoid higher ranked ones. The result is that the competition over limited resources may increase (Zeeb et al,. 1988; Bøe and Færevik, 2003).

Because of the inter-relatedness of stocking density, group size and pen size, separating the effects of each these three factors is a challenge. For example, when testing the effect of stocking density for pens of equal sizes, the number of animals must be manipulated, resulting in changes in stocking density and group size. One solution is to design experiments that consider the effects of stocking density, group size and pen size together (Leone and Estevez, 2008).

1.4. CHANGING STOCKING DENSITY AT REGROUPING

Changing density at regrouping happens in many domestic species. For example, under commercial conditions horses are frequently grouped with unfamiliar animals when moved to new locations, when being sold to new owners, or even when mixed with other horses while grazing for a part of the day. In these situations group members vary as well as the group size, both affecting social order and increasing aggressive competition among horses (Gonyou and Stricklin, 1998; Houpt and Keiper, 1982).

Two separate streams of studies have suggested that: 1) regrouping and changing densities independently have negative impacts on cows' welfare, and 2) the impact of an increase in density on the welfare of dairy cows would even be greater if this occurs at the same time as regrouping. However, no studies to date have tested the effect of changing density at the time of regrouping in dairy cattle. In addition, to the best of my knowledge no experiments to date have tested the effects of group size and pen size during grouping of any species (Bøe and Faerevik, 2003). The only related study, done on pigs (Hyun et al., 1998), reported that grouping and increasing density reduced growth rate and changed feeding behaviour. In this latter study the authors increased stocking density by reducing pen size but did not change the group size.

Some researchers have also suggested that stocking dairy cattle at higher densities at regrouping is the underlying reason for some emerging health problems in these animals. Nordlund et al. (2006) suggested that the increase in the incidence of ketosis after calving in recent years could be attributed to transition cows being frequently regrouped and overstocked in modern dairy systems. Changing density at regrouping may have suppressive effects on these animals' immune system by restricting their access to resources (feed, stall) and also by increasing competition over these resources. Behavioural changes such as increased competitive behaviour and decreased feeding time and lying time can be used as early indicators of dairy cattle welfare problems in response to changes in density at the time of regrouping (Nordlund et al., 2006).

1.5. DAIRY COW BEHAVIOUR

1.5.1. Feeding and Lying Behaviour

In recent decades scientists have described daily time budgets of dairy cows in free-stall systems. According to Grant and Albright (2000), a typical cow spends an average of 3 - 5 h / d feeding, 12 - 14 h / d lying down and 2 - 3 h / d engaged in social interactions in a free-stall environment. The remaining time is spent ruminating, drinking and outside the pen (e.g. milking). Changes in the typical time budget can be used as indicators of management, housing and health problems (Rushen et al., 2008).

It has been shown that feeding and lying behaviours of dairy cows are related. For example, dairy cows need to rest for a certain amount of time per day and are willing to give up some of their other behaviours (e.g. feeding) to achieve this rest requirement (e.g. Metz, 1985; Hill et al., 2009; Munksgaard et al., 2005). In a classic study, Metz (1985) showed that by restricting feeding and lying simultaneously, cows prefer to lie rather than eat. More recently, Munksgaard et al. (2005) showed that early and late lactating cows spend more time lying down than feeding under conditions of restricted access to stalls and feeders. In addition, in some studies, it has been shown that when overstocked, dairy cows prefer to rest rather than to eat after being milked (e.g. Hill et al., 2009). These studies show that there is a relationship between feeding and lying behaviour and stocking density and suggest that feeding and lying times are useful measures when assessing the effects of changes in stocking density.

1.5.2. Competition and Aggressive Behaviour

Competition is defined as "striving of two or more individuals to obtain a resource that is in limited supply" (Fraser and Broom, 2007). A competitive behaviour can be expressed in physical (e.g. butting, pushing) or non-physical ways (e.g. eye contact, threatening to butt, etc.). Aggressive competitive behaviour is described as the physical action of an animal to make another animal (usually lower ranked) withdraw from the resources (Fraser and Broom, 2007). Aggressive behaviour can cause fear, pain and injury and usually increases when resources are limited and animals are forced to compete. A displacement occurs when a cow's head or any other parts of her body comes into contact with another cow's body when it is using a resource (e.g. when feeding) causing her to withdraw from the resource (Huzzey et al., 2006; Fraser and Broom, 2007). Some studies have used competitive behaviour as a primary indicator of cow welfare in relation to changes in management and housing (e.g. Hasegawa et al., 1997; DeVries et al., 2004). Some of these studies also tested the effects of competition on other indicators of cow welfare such as feeding behaviour and lying behaviour (as secondary indicators of welfare) (e.g. Huzzey et al., 2006; Hill et al., 2009).

When spatial access to food and lying areas are decreased, the level of competition is expected to increase (Bøe and Færevik, 2003). Even in situations of unlimited access to feeding and resting resources, dairy cows may still compete for feeders and stalls but this level of competition is believed to have minimal effects on feeding and resting behaviours (Dado and Allen, 1994; Olofsson, 1999). It has been suggested that in free-stall systems the level of competition is higher at the feeding area than the lying area (Miller and Woodgush, 1991; Val-Laillet et al., 2008). The higher level of competition at the feeding area might be because cows are highly motivated to feed at the same time (i.e. after milking and when new feed is delivered; DeVries et al., 2003) and also because cows tend to move along the feeding area when eating, with the higher ranked cows preferring more space (Nordlund et al., 2006). Although feeding space measured for each cow varies among studies (e.g. 0.45 m feeding space / cow, Endres and Espejo, 2010; 0.52 to 0.56 m feeding space / cow, Caraviello et al., 2006), it is typically recommended that at least 0.6 cm of feed bunk should be provided for each cow to enable all cows to access feed simultaneously during peak feeding periods (e.g. after delivery of fresh feed; Grant and Albright, 2001; NFACC, 2009).

Although physiological mechanisms control feeding behaviour, it is also suggested that feeding behaviour is greatly influenced by the environment and feeding systems (Mertens, 1994). Olofsson (1999) showed that by reducing the number of feeders, competition at the feeding area increased, and feeding time and feed intake decreased. Zeeb et al (1988) also showed that competition at the feeding area is higher when the ratio of feeding places to animals is less than 1/1. Recent research has confirmed that competition at the feeding area is affected by the amount of feeding space available. For example, Huzzey et al. (2006) showed that as the stocking density increased at the feed bunk (from 0.81 to 0.21 m feeding space / cow) cows were displaced more often from the feeding area and as competition at the feed bunk increased feeding time decreased. In another example, DeVries et al. (2004) showed that as the stocking density decreased (from 0.5 to 1 m feeding space / cow) competition over feed decreased and feeding time increased. These studies also suggested that the effect of changes in density on feeding behaviour of dairy cows were more pronounced within 60 - 90 min after the delivery of fresh feed.

Some studies have tested the effects of regrouping of dairy cows on competitive behaviour. For example, Hasegawa et al. (1997) showed that regrouping disturbs social order in first-lactation heifers and increases competitive behaviour. Other studies have tried to show the consequences of increased competition on feeding and lying behaviour. von Keyserlingk et al. (2008) found that after regrouping, dairy cows increased competition at the feed bunk as well as decreased time spent lying. Schirmaan et al. (2011) showed that regrouping has negative effects on social behaviour, feed intake, feeding rate and rumination of dry cows (i.e. 3 months before parturition when cows are not milked).

1.5.3. Locomotor Activity

Cows walk when searching for food, water and resting areas, exploring the environment and avoiding threats (e.g. the aggressive behaviour of higher ranked animals). In social animals such as dairy cows, movements by some animals may elicit the movement of others (Fraser and Broom, 2007). Locomotor activity can also be affected by stocking density. For example, increasing stocking density decreases movements in broilers (Weeks et al., 2000); however, these changes are often accompanied by increased body weight and associated lameness may also restrict mobility in broilers (Newberry and Hall, 1990; Weeks et al., 2000). In another example, Leone and Estevez (2008) showed that as stocking density decreased, exploratory behaviour, distance travelled and the individual distance between animals increased resulting in increased movements of broilers within the pen.

It has been suggested that dairy cows decrease locomotor activity at higher densities simply because less space is available to move and lower ranked cows do not have enough space to escape the aggressive behaviour of higher ranked cows (e.g. Olofsson; 1999; DeVries et al., 2004). However, other studies have suggested that locomotor activity may increase at higher densities because increased competition forces cows to move to access resources or escape competitors (Zeeb et al., 1988; Bøe and Færevik, 2003). No study to date has tested the effect of stocking density (or regrouping) on locomotor activity in dairy cows.

1.6. RESEARCH OBJECTIVES AND HYPOTHESES

1.6.1. Objectives

The objectives of this study were to examine how changing stocking density at the time of regrouping affects: 1) competition as the primary indicator of cows' welfare, 2) feeding behaviour, 3) lying behaviour, and 4) locomotor activity as secondary indicators of welfare as a consequence of changes in competition. An additional objective was to assess these changes in terms of group size and pen size after accounting for the effect of density.

1.6.2. Hypotheses

I predicted that numbers of displacements at the feed bunk would increase on the day after regrouping as an indicator of increased aggressive competition over feed. When stocking density changed at the time of regrouping in our experiment, I expected competition to vary with the nature of the change in stocking density. For example, I expected that when stocking density decreased dairy cows would show less competition at the feeding area on the day after regrouping compared to the day before, but when stocking density did not change I expected cows to increase competition due to regrouping.

I also hypothesized that regrouping and changing density would affect lying behaviour and feeding behaviour. For instance, I predicted at the time of regrouping, increased stocking density would result in decreased lying time and feeding time. I also predicted that locomotor activity would change on the day after regrouping; for example, cows moved to higher density groups would increase locomotor activity.

I also expected that after accounting for the effect of density additional effects of changes in group size and pen size (as the forming factors of stocking density) would influence the outcome variables.

CHAPTER 2: INCREASED STOCKING DENISTY COMPOUNDS THE NEGATIVE EFFECTS OF REGROUPING ON SOCIAL BEHAVIOUR, LYING BEHAVIOUR AND LOCOMOTOR ACTIVITY OF DAIRY COWS

2.1. INTRODUCTION

In free-stall systems cows have the opportunity for social contact with other cows (Bøe and Færevik, 2003), but are often required to compete for access to resources including feed and stalls. Regrouping and changing stocking density are two management practices that increase this competition (e.g. Miller and Woodgush, 1991; Hasegawa et al., 1997; Olofsson, 1999), potentially compromising cow welfare.

On commercial farms, cows are frequently moved and regrouped to form groups similar in age, stage of lactation, milk production, health and reproductive status (Grant and Albright, 2001; Bøe and Færevik, 2003). Cows often experience four to five regrouping events during one lactation (Grant and Albright, 2001). Research on the effects of regrouping in dairy cows is minimal (von Keyserlingk et al., 2008), and some work on regrouping suffers from poor replication and limited sample size (e.g. Brakel and Leis, 1976; Hasegawa et al., 1997). Previous work suggests that mixing cows with unfamiliar animals that already have an established social order destabilize the social dynamic in the group (Bøe and Færevik, 2003). After regrouping, dairy cows increase physical competition (e.g. Kondo and Hurnik, 1990; Bøe and Færevik, 2003) and this increased competition can result in reduced lying and feeding time further compromising welfare (e.g. Philips and Rind, 2001; von Keyserlingk et al., 2008; Schirmaan et al., 2011).

Regrouping may also affect the stocking density within the pen, which also affects competitive encounters among cows (Bøe and Faerevik, 2003). Increasing stocking density can increase competition over feed, and decrease the time a cow spends feeding and lying down (e.g. Huzzey et al., 2006; Fregonesi et al., 2007).

Some authors have speculated that locomotor activity may increase at higher densities, because increased competition may force cows to move to access resources or escape competitors (Zeeb et al., 1988; Bøe and Færevik, 2003). Others have argued that dairy cows may decrease locomotor activity when housed at higher densities because less space is available and cows are unable to escape the aggressive behaviour of other cows (Estevez et al., 2007; Fraser and Broom, 2007). Research on chickens has shown that locomotor activity is affected by stocking density, group size and pen size (e.g. Leone and Estevez, 2008) but no study to date has tested the effect of stocking density (or regrouping) on locomotor activity in dairy cows.

Separate lines of experimental work suggest that both regrouping and increased stocking density have negative impacts on cows, but to date no study has assessed the combined effects despite that these practices are often combined on commercial farms. Thus the aim of this study was to evaluate competitive, feeding and lying behaviours and locomotor activity of dairy cows when being regrouped into pens with varying densities. We predicted that competition would increase when density increased at regrouping, which in turn would cause a reduction in feeding and lying time and an increase in locomotor activity.

2.2. MATERIALS AND METHODS

2.2.1. Animals, Housing and Diet

This experiment was performed at the University of British Columbia's Dairy Education and Research Centre (Agassiz, British Columbia, Canada) and used 72 lactating Holstein cows. Experimental cows were selected randomly from mid and late lactation cows in the herd with average parity of 2.6 ± 1.84 (mean ± standard deviation; range 1 to 9), average DIM of 204 ± 47 (range 125 to 296) and average milk production of 19 ± 3.5 kg (range 12 to 29.5). Cows were managed in compliance with the guidelines set by the Canadian Council on Animal Care (CCAC, 2009).

Cows were housed in either small pens (7.5 m * 13.5 m) with 12 stalls or large pens (15 m * 13.5 m) with 24 stalls, configured in 3 rows of 4 stalls (small pen) or 8 stalls separated by a cross-over alley (large pen; Figure 2.1.). Cows were fed a total mixed ration consisting of 27.2 % corn silage, 16.7 % grass silage, 8.5 % alfalfa hay and 47.6 % concentrate and mineral mix on dry matter (DM) basis. Major ingredients of concentrate include fine ground barley, rolled barley, rolled corn, distillers corn wheat blend, canola meal, and soybean meal. Feed was formulated based on National Research Council (NRC, 2001) recommendations. Water was available ad libitum. Milking took place twice a day from 5:00 a.m. to 6:00 a.m. and from 3:00 to 4:00 p.m. Fresh feed was delivered twice daily during each milking so that cows had access to the fresh feed when they returned to their pens.

2.2.1. Experimental Design

Two replicates were conducted each lasting five weeks; the first replicate took place in October and November 2009 and the second in April and May 2010. In each replicate, 24 cows were randomly selected as focal cows and 12 others as non-focal cows. Focal cows were randomly assigned to four groups of six cows each; non-focal cows were assigned to two groups of “filler cows”.

Three different stocking densities (25 %, 50 % and 100 %) were calculated based on a per cow access to a resource (feed bunk or lying stall) and by manipulating group size (6 versus 12 cows) and pen size (small versus large; Figure 2.2). Gates were moved to form small and large pens and six non-focal cows used to change the group size. Focal cows remained in the same pen throughout the experiment but non-focal cows were moved upon return from morning milking and grouped with focal cows to form the large groups (12 cows) and remained in the same pen for 7 days. All experimental pens were separated by non-experimental pens. Densities at the feed bunk were: 2.5 m / cow (25 %), 1.25 m / cow (50 %) and 0.63 m / cow (100 %) and at the lying stalls were 4 stalls / cow (25 %), 2 stalls / cow (50 %) and 1 stall / cow (100 %).

To assess the effects of different stocking densities at the same time as regrouping we examined the effect of decreasing density by a factor of 4 (100 % to 25 %), 2 (100 % to 50 % or 50 % to 25 %), or no change (50 % to 50 %) and increasing density by a factor of 2 (50 % to 100 % or 25 % to 50 %) or 4 (25 % to 100 %). For example, we increased number of cows from 6 to 12 and decreased lying stalls from 24 to 12 to increase stocking rate by a factor of 4 (25 % to 100 %; Figure 2.2.) or in the case of no change increased the group size from 6 to 12 and increased the pen size from 12 to 24 (50 % to 50 %).

Stocking density treatments were applied to groups of cows combined with a regrouping event using the design shown in Figure 2.2. Given the complex nature of the treatments we were not able to maintain equal number of replicates per treatment: each treatment was replicated 4 times with the exception of when density decreased or increased by a factor of 4 in which case there were only 2 replicates (see Figure 2.2.). Cows were regrouped every 7 days.

2.2.3. Data Collection

Pens were monitored 24 h / d using 16 digital cameras (WV-BP330, Panasonic, Osaka, Japan). Small and large pens had two and four cameras, respectively. Videos were recorded using a digital video surveillance system (GeoVision, version 8.3, GeoVision Inc, Corona, California). A red light (100 W) was placed beside each camera to improve the video quality at night. Each focal cow was dyed (see Huzzey et al., 2005) on its back and sides with individually distinct symbols to aid in identification.

Feeding and lying time were scored using scan sampling every 5-min from one day before to one day after regrouping. Cows were considered to be feeding when the neck collar was beyond the feed rail.

To record displacements, videos were watched continuously for 3 hours following the afternoon fresh feed delivery (from approximately 3:30 p.m. to 6:30 p.m.) one day before and one day after regrouping using Observer video-pro (version 5.0, Noldus Information Technology, Wageningen, Netherlands). A cow was recorded as the actor when she initiated the competitive displacement at the feed bunk which involved physical body contact with another cow's body while feeding, resulting in the other cow withdrawing her head from the feed rail (reactor).

Locomotor activity sensors (IceTag™ and IceTag™ 3D 1.008, IceRobotics Ltd, Midlothian, Scotland, UK) were attached to the lateral side of hind leg of each focal cow. Each week data were downloaded using software (IceTag Analyzer for IceTag™ and IceTag™ 3D 1.001/1.008, IceRobotics Ltd, Midlothian, Scotland, UK) programmed to extract data for every hour. In this study the term "number of steps" refers to the number of steps measured by IceTags. Tags were alternated between the two hind legs on alternate weeks for the duration of the experiment. Periods of time when cows were outside of the pen during milking were excluded from the final data set.

2.2.4. Data Analysis

Cows were treated as observational unit and pens as experimental unit. Inter-observer reliability was tested between the two trained observers (15 observations for each of feeding, lying and displacement variables) using regression and the results showed strong agreement ($R^2 > 0.98$).

Proc Univariate in SAS (version 9.2, SAS Institute Inc., Cary, North Carolina, USA) was used to evaluate distributions for normality and the presence of outliers. An observation was considered as an extreme outlier when it was more than three times outside the inter-quartile range; three observations for the displacements and one for the lying time were identified as outliers and excluded. Activity sensor recordings from three cows were also excluded due to missing values. One cow was in the estrus and was excluded from all analyses.

For all variables, data were summarized to create one observation per day before, and one day after each regrouping for each group. The means were used to calculate the difference in responses from the day before to the day after regrouping. This difference contributed to one observation for each group and treatment, a total of 32 observations. Proc Mixed in SAS was used to detect the effect of different stocking densities tested as a continuous effect (1 d. f.), group size (categorical, 1 d. f.), pen size (categorical, 2 d. f.) and the interaction between group and pen size (2 d. f.) on all dependent variables; these effects were tested in order using the htype=1 option in SAS. Pen and replicate were included in the model as random effects.

2.3. RESULTS

2.3.1. Competitive Behaviour

On the day before regrouping, focal cows engaged in displacements at the feeding area approximately 6 times / pen during the 3 hours after delivery of fresh feed in the afternoon. After regrouping the number of displacements changed depending upon the change in stocking density (Figure 2.3.; F = 10.48, d. f. =1, 18; P = 0.004). For instance, the number of displacements declined by approximately 8 events / 3 h when stocking density decreased by a factor 4 on the day after regrouping. In contrast, when stocking density increased by a factor 4, the number of displacements increased by approximately 14 events / 3 h.

After accounting for the effect of density, there was no additional effect of changes in group size or pen size or the interaction between group size and pen size on changes in the number of displacements at the feeder (Table 2.1). When stocking density remained stable the number of displacements did not change on the day after regrouping.

2.3.2. Lying Behaviour

The day before regrouping, focal cows averaged 13.5 h / d lying down and changes in this behaviour after regrouping again varied with changes in stocking density (F = 23.88, d. f. = 1, 18; P = 0.0001). For example, when density decreased by a factor of 4 at the time of regrouping, the amount of time cows spent lying down increased by approximately 1.2 h / d. Conversely when density increased by a factor of 4, lying time decreased by approximately 0.6 h / d. After accounting for the effect of denisty, the change in time spent lying down after regrouping did not vary with the change in group size, pen size or the interaction between group size and pen size. When stocking density did not change, lying time did not change on the day after regrouping.

2.3.3. Locomotor Activity

Focal cows averaged 444 steps / d on the day before regrouping. Changes in number of steps from one day before to one day after regrouping varied with changes in stocking density at regrouping (F = 4.47, d. f. = 1, 18; P=0.04). For example, when the stocking density decreased by a factor of 4 the number of steps declined by approximately 83 steps / d; however, where density increased by a factor of 4 the number of steps increased by approximately 17 steps / d. After accounting for the effect of density, changes in the number of steps after grouping did not vary with changes in pen size or the interaction between these factors. When stocking density did not change (i.e. group size and space size changed relatively), locomotor activity did not change on the day after regrouping.

2.3.4. Feeding Behaviour

On the day before regrouping focal cows spent approximately 5 h / d at the feeder. There was no change in this behaviour in relation to changes in stocking density at regrouping (F = 0.69, d. f. = 1, 18; P = 0.41). However, changes in feeding time after regrouping did vary with changes in group size (F = 4.75, d. f. = 1, 18; P = 0.04). For example, when group size decreased by a factor of 2 (from 12 to 6 cows) at the time of regrouping, feeding time decreased by approximately 0.4 h / d. Conversely, when the group size increased by a factor 2 (from 6 to 12 cows) feeding time increased by approximately 0.5 h / d. There was no effect of changes in pen size or the interaction between group size and pen size. Even when stocking density did not change, feeding time changed on the day after regrouping.

2.4. DISCUSSION

2.4.1. Competitive Behaviour

On the day before regrouping, focal cows were involved in approximately 8 displacement encounters during the 3 hours after delivery of fresh feed. This value is similar to the results of other studies on competitive behaviour in dairy cows (e.g. 10 times / 3 h; von Keyserlingk et al., 2006). Results of this study indicate that aggressive competition over resources increases after regrouping and supports findings of previous studies (e.g. Brakel and Leis, 1976; Hasegawa et al., 1997; von Keyserlingk et al., 2008). Regrouping disturbs social behaviour in other farm animals including pigs (e.g. McGlone and Curtis, 1985; Stookey and Gonyou, 1994) and laying hens (e.g. Abeyesinghe et al., 2009). It is well known that when stocking density increases, competition among cows at the feeding area also increases (e.g. Olofsson, 1999; DeVries and von Keyserlingk, 2006). Similar studies in calves (Kondo et al., 1989) and transition cows (e.g. Proudfoot et al., 2009) also show that increasing stocking density increased aggressive competition over feed. Results of our study agree with these previous studies showing that increased stocking density at regrouping increased the number of displacements at the feeding area during the 3 h following fresh feed delivery when cows are highly motivated to feed (e.g. DeVries and von Keyserlingk, 2005).

On dairy farms stocking density often changes at the time of regrouping. Although previous work has studied the impact of changes in stocking density on the welfare of dairy cows and other work has investigated the impact of regrouping, to date no studies have tested the effects of changes in density when cows are regrouped. The current study addresses this gap in the literature by showing that regrouping and changing stocking density both have negative impacts on the cow. Results of our study indicate that changes in the number of displacements at the feed bunk after regrouping varied with the change in stocking density (i.e. when stocking density increased number of displacements increased on the day after regrouping, and when stocking density decreased this measure declined). Moreover, to our knowledge, no experiment to date has tested the effects of group size and pen size during regrouping in any species. The only related study, which was done on pigs (Hyun et al., 1998), reported that grouping and reducing pen size reduced growth rate and changed feeding behaviour.

2.4.2. Lying Behaviour

On the day before regrouping, cows in the current study spent approximately 13.5 h / d lying down; this value is within the range found by other studies (12 - 14 h / d; e.g. DeVries and von Keyserlingk, 2006; Huzzey et al., 2006). Results of the few studies available on the effects of regrouping and stocking density on lying behaviour of dairy cattle suggest that cows spend less time lying down due to increased competition after regrouping (e.g. Hasegawa et al., 1997; Philips and Rind, 2001; von Keyserlingk et al., 2008) and increasing stocking density (e.g. Fregonesi et al., 2007; Hill et al., 2009; Proudfoot et al., 2009).
Studies on the effects of regrouping and changing densities on lying behaviour of dairy cows indicate that these practices can cause dairy cows to spend less time lying down. Our results are consistent with these previous studies, and also indicate that regrouping *and* stocking density together influence lying behaviour and more specifically indicate that changes in lying behaviour due to regrouping are dependent upon changes in stocking density. Our

results showed that lying time decreased when stocking density increased and increased when stocking density decreased.

2.4.3. Locomotor Activity

The current study was the first to test the effects of stocking density and regrouping on the locomotor activity in dairy cows. Previous studies have used increases in the number of steps to detect estrus in dairy cattle in order to predict ovulation time and improve success of artificial insemination (e.g. Arney et al., 1994; Maatje et al., 1997). Though there is no published research available on the effects of stocking density on locomotor activity of adult dairy cows, some authors have suggested that locomotor activity may increase at higher densities because of higher numbers of competitive encounters (Zeeb et al,. 1988; Bøe and Færevik, 2003). To our knowledge the only related paper on this topic is the study by Veissier et al. (2001) who reported increased locomotor activity in calves after regrouping.

Changes in the number of steps from one day before to one day after regrouping varied with the changes in stocking density at regrouping; as stocking density increased at regrouping numbers of steps increased. These results agree with Zeeb et al. (1988) and Bøe and Færevik (2003) who suggested that increased competition due to increased density may force cows to compete for access to resources, or escape competitors, leading to an increase in locomotor activity. Increased locomotor activity after increasing stocking density at regrouping may also relate to the reduced lying time. Ice-tags data was downloaded during the period between morning and afternoon milking on the day after regrouping in first replicate. I expect that if this period was included the change in number of steps would be more prominent.

2.4.4. Feeding Behaviour

On the day before regrouping focal cows spent approximately 5 h / d at the feeding area. This result is similar to previous studies indicating that dairy cows spend an average of 3 - 5 h / d feeding (e.g. DeVries and von Keyserlingk, 2006; Huzzey et al., 2006). The few studies available on the effects of regrouping on feeding behaviour of dairy cows suggest that this practice may negatively impact feeding behaviour due to changes in social order and increased competitive behaviour (e.g. Hasegawa et al., 1997; Philips and Rind; 2001). Decreases in feeding time in response to increased stocking density at the feeding area, likely occur as a result of the increase in competitive interactions over feed (e.g. Olofsson, 1999; DeVries et al., 2004; Huzzey et al., 2006). We predicted that feeding time would decrease as the stocking density increased at regrouping, but we found no consistent change in this behaviour. Some previous studies on regrouping and stocking density also found no effects of these practices on the time dairy cows spent feeding. For example, Hill et al. (2009) found that increasing density decreased lying time but did not affect feeding time. von Keyserlingk et al. (2008) found that regrouping caused dairy cows to increase competition over feed, but not change time spent feeding on the next 3 days after regrouping.

Why have some studies demonstrated an effect of changing density and regrouping on feeding time while others have not? One explanation may be that the studies that demonstrated effects changed stocking density only by restricting feeding space and did not change pen size or number of stalls (e.g. Olofsson, 1999; DeVries et al., 2004; Huzzey et al., 2006). In contrast, studies that did not demonstrate the effects of stocking density on feeding behaviour restricted access to both lying stalls and feeding area. Previous studies suggested that cow are willing to give up some of their behaviours (e.g. feeding) in order to lie down during situations of having limited access to these resources (e.g. Metz, 1985; Hill et al., 2009; Munksgaard et al., 2005). We speculated that when stocking density increases at the feeding area some cows may give up feeding and leave the feeding area to lie down. Such situations may result in a reduction in the average time spent feeding for the pen. When both lying stalls and feeding space are restricted, those animals are also less able to lie down and spend more time standing. It is likely that some of this time will be spent in the feeding area thus reducing the chance of finding differences in feeding time.

Group size and pen size both affect stocking density, but work examining the effects of group size and pen size is minimal and the results to date are contradictory (e.g. Kondo et al., 1989). Furthermore, to date no experiments have measured the effects of group size and pen size during regrouping and changing density in dairy cows (Bøe and Faerevik, 2003). Because of the inter-relatedness of stocking density, group size and pen size separating the effects of each of these three factors is a challenge (Leone and Estevez, 2008). Our experimental design enabled us to test the effects of group size and pen size on the outcome variables. Results showed that after accounting for the effect of density, only one of the behavioural measures varied with group size; when group size decreased feeding time decreased and when group size increased feeding time increased. It has been shown that by increasing the number of male lambs in a pen, time spent feeding increases (Jenkins and Leymaster, 1987). This increase in feeding time may be because animals follow the behaviour of other animals (i.e. social facilitation; e.g. Cooper et al., 2008).

2.5. CONCLUSIONS

Two separate lines of evidence have demonstrated that regrouping and increased stocking density increase competition and affect feeding and lying behaviour. The current study was the first to show the effects of these two management practices in combination.

Our findings indicate that by increasing stocking density at regrouping, aggressive competition over feed increases and this behavioural change interferes with the daily time budget of cows leading to decreased lying time and increased locomotor activity. Our results indicate that famers may be able to alleviate the negative effects of regrouping on behaviour and welfare of dairy cows by reducing stocking density. Our experimental design considered group size and pen size in addition to stocking density and showed that changes in feeding time after regrouping varied with the change in group size independent of density.

2.6. ACKNOWLEGEMENTS

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Figure 2.1. Layout of a large experimental pen; separation gates were used to create the small pens.

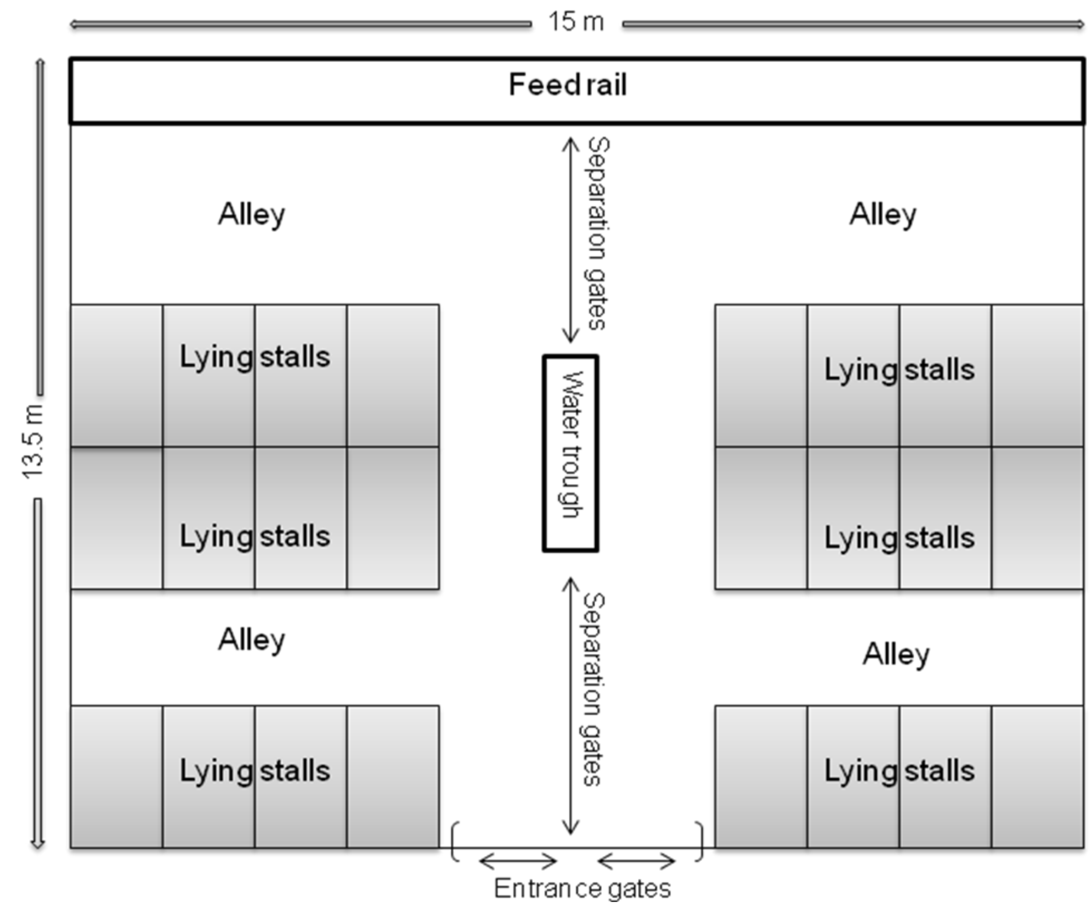

Figure 2.2. Time course of change in pen size and group size used to create different densities in each replicate. Large white rectangles show the large pens and small white rectangles show the small pens. Numbers within boxes show the number of cows in each pen. Arrows show change in densities imposed to each regrouping.

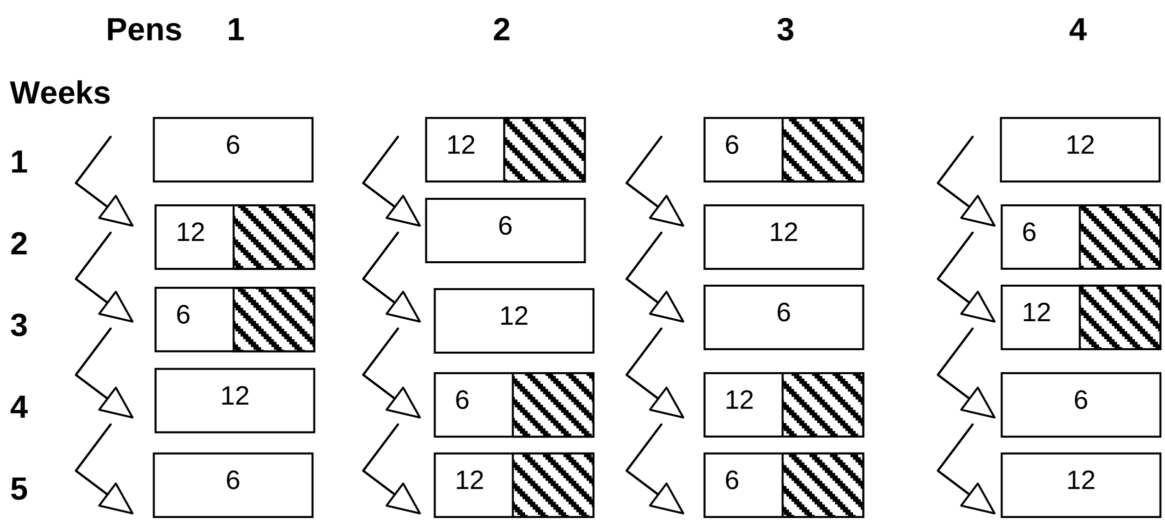

Figure 2.3. Behavioural differences from one day before to one day after imposing changes in stocking density. Means (± S.E.M.) are shown separately for: displacement events in feeding area (a) time spent on lying (b) and, number of steps (c). Change in density varied from decrease density by a factor 4 and by a factor 2, no change in density (0), increase density by a factor 2 and by a factor 4.

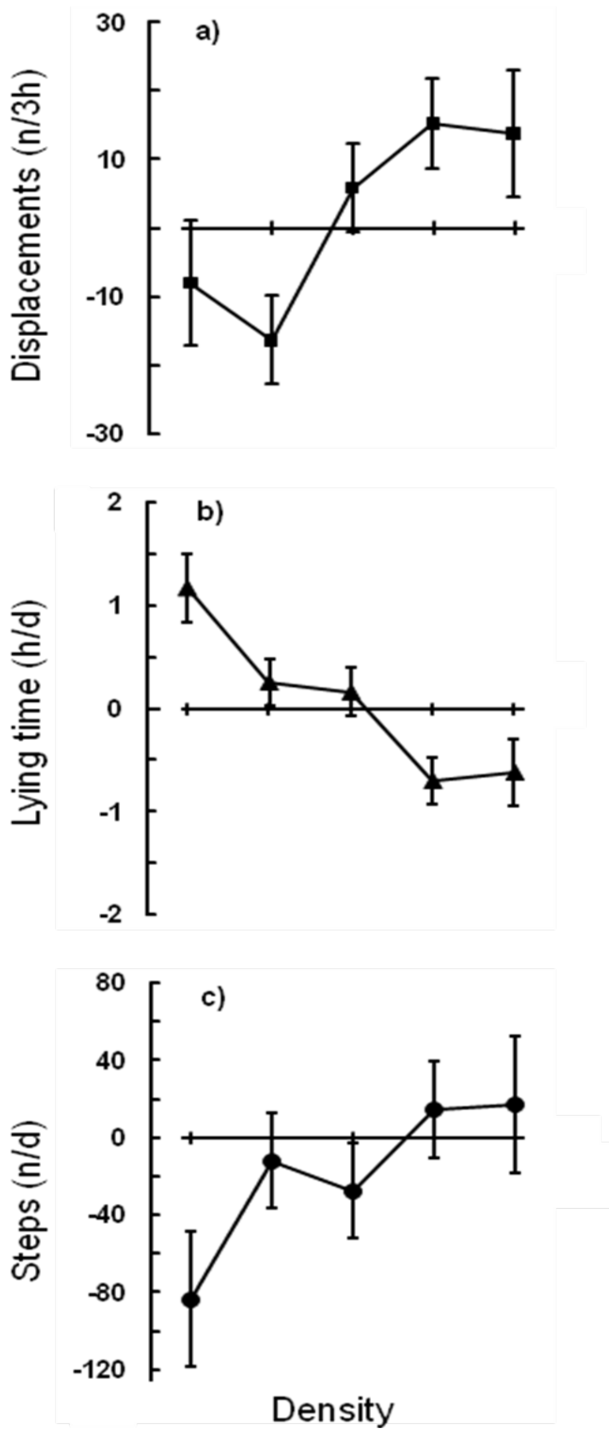

Table 2.1. Least Square Means ± SE of response variables calculated for the interactions between the levels of change in group size and pen size. Group size has increased by a factor 2 (6 to 12 cows) and decreased by a factor 2 (12 to 6 cows). Pen size has increased by a factor 2 (12 to 24 stalls), did not change (from 12 to 12 or from 24 to 24 stalls) and decreased by a factor 2 (from 24 to 12 stalls). P-values are for tests of the effects of group size, pen size and the interaction between group size and pen size.

	Changes in group size and pen size						***P-values***		
	Group size ↑			***Group size ↓***					
Changes in:	***Pen ↑***	***Pen =***	***Pen ↓***	***Pen ↑***	***Pen =***	***Pen ↓***	***Group size***	***Pen size***	***Goup*Pen***
Displacements (#/3h)	-12±11	4±9	-9±11	9±11	9±7	2±05	0.23	0.45	0.66
Lying time (h)	-0.2±0.4	0.1±0.3	-0.4±0.4	-0.5±0.4	0.2±0.3	0.4±0.5	0.96	0.19	0.26
Steps (#)	57±41	2±33	6±41	-12±41	-50±27	-70±56	0.23	0.24	0.92
Feeding time (h)	0.5±0.4	0.6±0.3	0.8±0.4	-0.1±0.4	-0.3±0.3	0.3±0.6	0.04	0.57	0.90

CHAPTER 3: GENERAL DISCUSSION

3.1. CONTRIBUTIONS AND IMPLICATIONS

Regrouped cows need to cope with two major challenges: 1) being mixed with unfamiliar cows and 2) being grouped in pens at higher densities. Both of these challenges can restrict access to resources, increasing aggressive competition. Moreover, if stocking density increases at regrouping these effects may be more severe. In contrast, when the spatial access to resources increases at the time of regrouping this may allow cows (especially lower ranked cows) to avoid aggressive encounters and have better access to the resources. Aggressive competition over resources can be used as a primary indicator that welfare is compromised. Increases in competition can reduce lying and feeding time and affect locomotor activity, further compromising cow welfare. Therefore the focus of this study was to determine how regrouping and stocking density together affect competition, lying, feeding behaviours and locomotor activity of dairy cattle.

In this study I examined stocking densities 100% and lower (25%, 50%); cows were not overstocked. The majority of previous studies have focused on the effects of overstocking (i.e. less than one stall or less than 60 cm feeding area per cow) on dairy cattle behaviour and welfare (e.g. Huzzey et al., 2006; Fregonesi et al., 2007). Overstocking may accentuate behavioural responses resulting in greater differences. In addition, the majority of studies have changed stocking density by blocking off stalls or feeding positions. One of the advantages of my experimental design was that I changed stocking density by changing the pen size, including the numbers of stalls and feeding space per animal. This method of changing density makes my results more applicable to commercial dairy farms where changes in stocking density typically affect access to both lying and feeding spaces.

3.2. LIMITATIONS AND FUTURE RESEARCH

Although outcomes of this study contribute to the literature on regrouping and stocking density, there were some limitations that should be addressed in future research. One limitation was that feeding time was scored from video using 5-min scan sampling. A cow was scored as “feeding” when her head was beyond the neck rail, but the camera images are still in each scan and I could not be certain if the cow was actually consuming food. Another limitation with this method is that behaviour was not measured during the interval between each scan. Hill et al. (2009) used 10-min scan sampling to score feeding time and did not find any effect of increased stocking density. Some previous studies (e.g. Schirmann et al., 2011; Proudfoot et al., 2009; Olofsson, 1999), investigating the effects of stocking density on feeding time, used automatic feed monitoring systems that provide more precise and continuous measures of feeding behaviour. These automatic recording systems are also able to measure feeding rate and feed intake. For example, Schirmann et al. (2011) showed that regrouping has negative effects on feed intake and feeding rate. I suggest that future work on regrouping and stocking density use automatic feed recording systems to measure feeding behaviour.

Continuous analysis of videos for recording physical encounters (i.e. displacements) is often used to measure competitive behaviour in dairy cattle. However, competitive behaviour can be expressed in physical (e.g. butting, pushing) or non-physical ways (e.g. eye contact, threatening to butt, etc.; Fraser and Broom, 2007). While scoring the competitive behaviour, I observed some non-physical interactions that resulted in the reactor cow withdrawing from the feed bunk. Unfortunately I was not able to reliably score these interactions as most of these non-physical competitive encounters cannot be seen using video. I therefore recommend that future studies incorporate some live monitoring to record these interactions. Live monitoring would also provide the opportunity to observe additional behaviours, such as vocalization that might be associated with competitive behaviour.

Changes in behaviour of dairy cattle after regrouping and changes in density may be associated with multiple factors such as composition of the group (age, social order, weight; e.g. Hindhede et al., 1999; Veissier et al., 2001), previous social experiences of the animals (Jensen et al., 1997; Veissier et al., 1994; Mench et al., 1990), and duration of regrouping (Hasegawa et al., 1997; Kondo et al., 1984). I recommend that future research on the effects of regrouping and stocking density take these factors into account. For example, it has been shown that social behaviour in dairy cows is affected by early social experience including different rearing methods (single crate or group rearing; e.g. Jensen et al., 1997), visual interaction with other calves (Arave et al., 1992), separation from dam (e.g. Veissier and Le Neindre, 1989). Thus future studies should focus on the role of early social experience on how adult dairy cattle cope with regrouping to higher densities later in life.

Compared with previous research (e.g. Brakel and Leis, 1976; Hasegawa et al., 1997; Philips and Rind, 2001) on regrouping and stocking density I used relatively large groups and pen sizes in my experiment, but these are typically much greater on commercial farms where group sizes in excess of 300 cows are common. I thus recommend that future work on this area also include work done on commercial farms. For example, competitive behaviour can be compared between farms using different regrouping strategies.

The outcomes of this study can be used in setting science-based standards for management, housing and feeding systems, ideally helping to prevent welfare problems at dairy farms. The results of my study showed that by reducing stocking density the negative impact of regrouping on cows can be reduced. Thus one recommendation to farmers is to minimize the number regroupings, and to avoid increasing stocking density when regrouping is necessary.

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