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Group management of young dairy cattle in relation to animal behaviour and welfare

Doctoral Dissertation

Satu Raussi

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Group management of young dairy cattle in relation to animal behaviour and welfare

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Abstract

This work consists of two collaborative research projects between INRA (France) and MTT (Finland) that examine the influence of group management on young cattle. The first work investigated whether pair versus individual housing of calves reduces their chronic stress reactions and whether positive contacts with humans could partly compensate for a lack of contact with conspecifics. In the second work, pair-housed heifers were either repeatedly regrouped or kept with their familiar peer. The consequences of repeated regrouping on heifers' social behaviour, emotional reactivity, physiology and production were analysed.

Calves housed in pairs seem less stressed than calves housed individually, and regular positive contacts with a stockperson can not compensate for the lack of social partners. Pair-housed calves are less ready than their individually housed counterparts to approach humans. However, positive contacts with the stockperson make calves less fearful of people and improve handling both in the individual and pair-housing.

Heifers housed in pairs and repeatedly regrouped are more aggressive between each other than heifers kept with the same penmate. However, repeated regrouping lowers heifers' behavioural reactivity in comparison with rearing heifers in stable pairs. Therefore, diversity in the social environment rather than stability appears to be more advantageous for heifers.

In conclusion, group housing is beneficial for the welfare of calves and a variety of social experiences with conspecifics offers advantages for heifers. Cattle of different ages seem to have different social needs that must be fulfilled to ensure their welfare.

Key words: behaviour, behavioural tests, calf, cattle, handling, heifer, human-animal interaction, social environment, stress, welfare

Nuorten nautojen käyttäytyminen ja hyvinvointi ryhmäkasvatuksessa

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Tiivistelmä

Kotieläinten käyttäytymis- ja hyvinvointitiedon tarve on jatkuvasti kasvanut. Kasvava tiedontarve liittyy lypsykarjataloudessa yksikkökoon suurenemiseen; eläimiä pidetään yhä useammin ryhmissä ja yhden hoitajan vastuulla on yhä suurempi määrä eläimiä. Tämä väitöskirja tarjoaa tietoa nuorten nautojen sosiaalisen ympäristön sekä ihmiskontaktin vaikutuksista eläinten käyttäytymiseen ja hyvinvointiin.

Väitöskirjatyö perustuu kahteen eläinkokeeseen, jotka tehtiin ranskalaisen INRA:n ja MTT:n yhteistyönä. Ensimmäisessä kokeessa tutkittiin, ovatko pareittain kasvaneet vasikat vähemmän stressaantuneita kuin yksilökarsinassa kasvaneet ja voiko positiivinen kontakti hoitajan kanssa osittain korvata lajitoverin seuran vasikoilla. Toisessa kokeessa tutkittiin toistuvan ryhmittelyn vaikutusta pareittain kasvatettujen hiehojen sosiaaliseen käyttäytymiseen, reaktiivisuuteen, stressifysiologiaan ja kasvuun.

Pareittain kasvatetut vasikat ovat yksilökarsinassa kasvatettuja vähemmän stressaantuneita, mutta hieman vaikeampia käsitellä. Hyväkään ihmiskäsittely ei korvaa lajitoverin seuraa, mutta positiivista kontaktia hoitajalta saaneet vasikat lähestyvät ihmistä nopeammin kuin minimikontaktia saaneet vasikat. Hyvä ihmiskäsittely on tehokasta sekä pari- että yksilökarsinassa kasvaneille vasikoille.

Toistuvasti ryhmitellyt hiehot ovat aggressiivisempia toisilleen verrattuna hiehoihin, jotka ovat vasikasta saakka kasvaneet yhdessä. Toistuva ryhmittely vaikuttaa kuitenkin hiehojen reaktiivisuuteen vähentävästi verrattuna samojen parien kanssa kasvaneisiin hiehoihin.

Ryhmäkasvatus edistää vasikoiden hyvinvointia ja ryhmittelyn tuomasta sosiaalisesta kokemuksesta on hyötyä hiehoille. Naudoilla on eri ikäkausina erilaisia sosiaalisia tarpeita, jotka tulisi tuotanto-olosuhteissa huomioida.

Avainsanat: hieho, hyvinvointi, ihmisen ja eläimen välinen suhde, käsittely, käyttäytyminen, käyttäytymistestit, nauta, sosiaalinen ympäristö, stressi, vasikka

List of original articles

The thesis is a summary and discussion of the following articles, which are referred to by their Roman numerals:

I S. Raussi, B.J. Lensink, A. Boissy, M. Pyykkönen, I. Veissier, 2003. The effect of contact with conspecifics and humans on calves' behaviour and stress responses. Animal Welfare 12, 191-203.

II B.J. Lensink, S. Raussi, X. Boivin, M. Pyykkönen, I. Veissier, 2001. Reactions of calves to handling depend on housing condition and previous experience with humans. Applied Animal Behaviour Science 70, 187-199.

III S. Raussi, A. Boissy, E. Delval, P. Pradel, J. Kaihilahti, I. Veissier, 2005. Does repeated regrouping alter the social behaviour of heifers? Applied Animal Behaviour Science 93, 1-12.

IV S. Raussi, A. Boissy, S. Andanson, J. Kaihilahti, P. Pradel, I. Veissier, 2005. Repeated regrouping of pair-housed heifers around puberty affects their behavioural reactivity. Animal Research. Accepted.

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Contents

1 Introduction: the challenge of group management of cattle

Group housing of cattle is a much discussed topic in Europe for two reasons. One is increased public concern for welfare in animal husbandry; another is European legislation (EC Report on the Welfare of Calves, 1995; Council Directive 97/2/EC) regarding the welfare of calves. Group housing is now compulsory in the European Union for calves over 8 weeks of age (Council Directive 97/2/EC). Because cattle are highly social animals (for review: Bouissou et al., 2001), housing them in groups instead of individually can improve their welfare. Cattle may, however, be kept individually in pens if they are under 8 weeks of age, if regulations are lacking to protect the welfare of farm animals outside Europe, or if animals are used for experimental purposes.

Cattle develop strong and long-lasting affiliative relationships with each other. These relationships are especially significant between relatives but also between animals that are kept together for the first months of life (Bouissou et al., 2001). Social relations between animals have a calming effect by reducing the impact of stressful conditions (Boissy and Le Neindre, 1990). Signs of strong relations between pairs of cattle are synchronised activities, tolerance during feeding competition and a high level of social licking (Veissier et al., 1990; Sato et al., 1993).

Although extensively reared beef cattle are maintained in near natural feral groupings before weaning, dairy calves are separated from the dam soon after birth (within a day to several weeks of birth) and usually reared in groups with other calves born during the same period. Heifers born in the same year are generally reared together until first calving, after which they are integrated into the main dairy herd, where they are subjected to further regroupings according to milk yield or production stage (Arave and Albright, 1981; Konggaard et al., 1982). Some heifers may also be sold. In contrast to feral cattle, young dairy animals often undergo many changes in their social environment. Mixing or regrouping has negative consequences on the welfare of cattle, affecting their behaviour and production (Hasegawa et al., 1997; for review: Bøe and Færevik, 2003).

Cattle farms in Northern Europe are becoming larger and the number of animals per unit is increasing, having an impact on human–cattle interactions. One stockperson is responsible for a growing number of animals and their welfare. New technology may change the character of husbandry tasks, which may in turn reduce human–animal interactions. In group housing as compared with individual housing, it is easier for cattle to avoid contact with humans because of more available space. This may lead to inadequate habituation of cattle to humans.

Defining animal welfare is as complex as measuring it. Therefore, many definitions exist. A very broad definition had been given by Huges in 1976: welfare is a state of mental and physical health where the animal is in harmony with its environment. What harmony means is not clear from this definition. According to Broom (1991), animals face environments which are more or less friendly; when an animal can find low-cost solutions to cope with the environment, its welfare is ensured; when larger efforts are needed, then its welfare is at stake. Hence, Broom (1991) defines welfare in terms of coping. However, welfare is more than good physical functioning, also refering to the mental state of animals (Duncan, 2002). Definitions thus should also include animals being sentient, i.e. capable of feelings (Webster, 1994). Welfare can therefore be defined as the absence of negative emotions such as fear, pain and frustration (Dawkins, 1983). One potential indicator of animal welfare is to measure an individual's stress responses. Although no clear definition of stress exists and links between welfare and stress are unclear, Moberg (2000) defines stress as a biological response elicited when an individual perceives a threat to its homeostasis. An animal can cope with stress by its behaviour and neuroendocrine, immunological and autonomic nervous system responses (Moberg, 2000). Stress is a part of normal life, and the challenge is to determine when stress becomes distress (the biological cost of stress), which has deleterious effects on the welfare of animals (for review: Moberg, 2000). For the purpose of this thesis, welfare will be defined as the quality of life experienced by an animal (Bracke et al., 2001). Perceived quality of life of an animal can only be indirectly assessed by its behaviour and physiological indices of stress, production and health.

Behaviour is a sensitive measure of animal welfare, probably more sensitive than animal health or production. Behaviour is elastic and easily modified in stressful conditions. In observing the behaviour of cattle, if action is taken at the first signs of distress, the situation can often be rectified before it becomes more serious. Changes in the time budget of an animal may, for instance, serve as a marker of change in welfare. For instance, weaning alters time spent standing or moving and the circadian rhythm of activity in heifers (Veissier et al., 1989), and repeated regrouping changes calves' daily rhythm of activity (Veissier et al., 2001). Behavioural responses of animals to novelty, suddenness and predator cues are measured to evaluate emotional reactivity (Boissy et al., 2001; Désire et al., 2002; 2004). Repeated regrouping enhances calves' behavioural reactivity to novelty (Boissy et al., 2001). Animals' fear of humans is measured by approach or avoidance behaviour to unfamiliar or familiar persons, with fearful animals typically avoiding contact with humans (Hemsworth and Coleman, 1998).

Cardiac and hypothalamic-pituitary-adrenal (HPA) axis activity measures have been widely used to study animals' physiological stress responses. Heart rate increases in response to excitement and physical restraint (Hopster and Blokhuis, 1994; Waiblinger et al., 2004). Cortisol responses to exogenous adenocorticotropic hormone (ACTH) and corticotropin-releasing factor (CRF) challenges have been reported to be more accurate measures than plasma basal cortisol concentration for long-term physiological stress in animals (Ladewig and Smidt, 1989).

In this thesis, we investigated how grouping of young cattle can affect their welfare; welfare was assessed by the behaviour of animals (time budget and reactivity) and their physiological stress responses. In addition, we analysed the consequences of grouping on subsequent reactions to conspecifics and to people.

2 Literature survey

2.1 Social behaviour of cattle

Farm animals are all social creatures with specific social organisations. Cattle are gregarious animals and synchronisation of foraging and resting behaviour is typical of this species (Bouissou et al., 2001).

Cattle use vocalisation to express excitement and interest in a situation and also to show frustration and stress, e.g. when isolated from conspecifics (Bouissou et al., 2001). Olfactory communication is important for social life and for individual recognition of species companions (Bouissou et al., 2001). Spraying cows with aniseed oil has been shown to reduce aggressiveness after grouping (Cummins and Myers, 1991). Olfactory bulbs and the vomeronasal organ are used, and cattle are able to relay odours directly to the vomeronasal organ. This is possible by presenting a special facial expression, called a flehmen response (for review: Albright and Arave, 1997). Cattle can communicate their physiological states by pheromones, especially when they are frightened and stressed (Boissy et al., 1998). Body language is important, especially the head position relative to the body, during aggressive and submissive displays (Schloeth, 1958).

Social interactions can roughly be divided into agonistic and non-agonistic encounters. Agonistic interactions include aggressive acts and responses to aggression, mainly avoidance or flight. Non-agonistic interactions include allogrooming (social licking) and sexual behaviour (Bouissou et al., 2001). The dominant animal will butt its opponent in the side or rump if the threatened animal is too slow to submit or fails to notice the threat. In a well-established hierarchy, the threatened animal will spontaneously retreat and take a submissive posture, with its head held low and directed away from the opponent (Bouissou et al., 2001). Before dominance relationships are established, fighting may occur. Fighting is displayed by head-to-head, followed by head-to-neck combat (Bouissou, 1985). Most of the fights are short – 80% last less than one minute – but the duration can vary from a few seconds to one hour (Bouissou, 1974).

After puberty, dominance-related behaviours and adult-type agonistic interactions, such as butting and threatening, become more prevalent (Bouissou, 1977). Bouissou (1985) has shown that dam-reared calves establish dominance relationships earlier than calves artificially reared (at 4-5 months vs. 9 months of age). Six-month-old heifers, previously unfamiliar to each other, are able to establish stable dominance relationships (Bouissou and Andrieu, 1978). According to Bouissou and Andrieu (1978), heifers are less aggressive towards former group members than unfamiliar animals at regrouping. Dominance relations between adult females are very stable, whereas relations between young animals or between males are less stable (Reinhardt and Reinhardt, 1975).

The position of an animal in the group hierarchy affects its maintenance activities. Low-ranking cows prefer to eat apart from high-ranking peers (Manson and Appelby, 1990). Protection of the head of a cow while feeding helps to increase the feeding time of low-ranking cows (Bouissou, 1970). High-ranking animals choose the best cubicles to lie down (Friend and Polan, 1974). The resting time of low-ranking animals may therefore be reduced (Bouissou, 1985).

In addition to dominance hierarchy, the social organisation of cattle is characterised by the affinity bonds holding the group together (Arnold, 1985). Social licking is an indicator of formation and maintenance of social bonds among cattle, and a high level of social licking is a sign of strong social bonds (Sato et al., 1993). This allogrooming may reduce tension, reinforce social bonds and stabilise social relationships (Sato et al., 1993). It is mainly directed to the head, neck and shoulder areas (Bouissou, 1985), whereas licking of the rump and anogenital areas is more often associated with sexual behaviour. Social licking is frequently preceded by a solicitation to be licked (Bouissou, 1985). All animals in the group are licked, but only 75% of individuals lick others (Sato, 1984). A high frequency of licking between two animals is also associated with closer physical proximity and higher synchronisation of activities (Veissier et al., 1990).

In calves, affinity bonds are strongly influenced by how long animals have been together (Ewbank, 1967; Reinhardt and Reinhardt, 1982). These bonds probably develop before the calves are six months of age (Bouissou and Andrieu, 1978). Bonds are stable; early peers prefer each other for at least one year (Bouissou and Andrieu, 1978; Reinhardt and Reinhardt, 1982). Preferred peers tend to rest and eat together, and they can better tolerate feeding competition than peers that are mixed later (Bouissou and Hövels, 1976). Animals of similar age or neighbouring rank or related animals prefer each other (Reinhardt, 1981) (Figure 1).

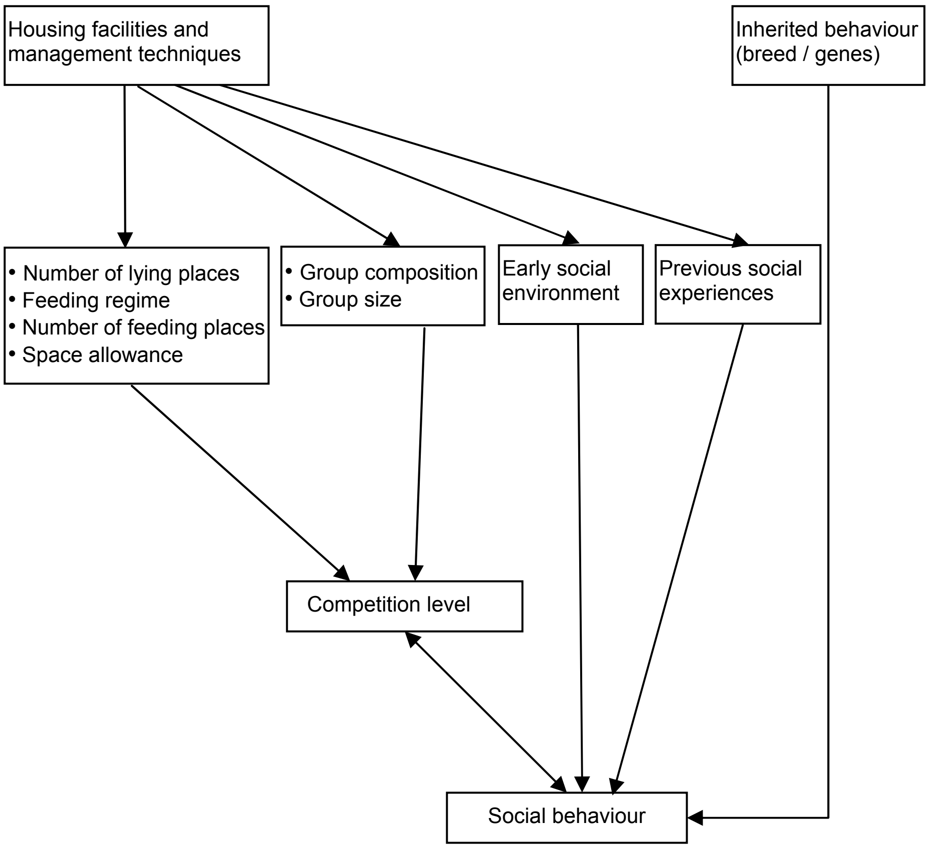


Figure 1. Factors that influence the social behaviour of cattle (adapted from: Bøe and Færevik, 2003).

Feral cows, heifers and calves (female and male) cohabitate within a large matriarchal herd of about 20 individuals, and new members are rarely accepted into the established group. Aggressive behaviour, such as fightings, is rare. Outside the mating season, males live in small male –only groups (Bouissou et al., 2001).

The social behaviour of cattle varies with age and maturity. Calves are hiders for the first 2-5 days after birth; that is, they stay hidden with their mother before joining the herd (Hall, 1986; Vitale et al., 1986). However, at three weeks of age, they spent most of their time with other calves (Le Neindre, 1984). In the first two months of life, calves have a few aggressive interactions, which tend to be more playful and bi-directional and do not result in a clear social hierarchy (Reinhardt and Reinhardt, 1982; Canali et al., 1986; Bouissou et al., 2001). Observations in a semi-wild (*Bos indicus*) herd of cattle indicate that play fighting occurs in calves at two weeks and social licking at four weeks of age (Reinhardt and Reinhardt, 1982). This play or ''mock fighting'' is displayed in different social contexts than the actual fights occurring among adults and ends abruptly with no specific consequences (Reinhardt and Reinhardt, 1982). Calves are not particularly aggressive towards each other after grouping compared with semi-mature or adult cattle. Veissier et al. (2001), for instance, reported less than two aggressive interactions between calves (from 5 to 18 weeks of age) during the first three hours following regrouping, whereas Bouissou (1974) observed ten aggressive interactions of heifers (at 18 months of age) within the first hour of regrouping. Calves also habituate to repeated regrouping, and thus, are less and less agitated when regrouping is repeated (Veissier et al., 2001).

2.1.1 Humans in the social environment of cattle

In some circumstances, the stockperson has been speculated to act as a substitute for social partners to calves (Arave et al., 1985). This is supported by the observation that lambs that have been reared alone and have received positive contacts, either by hand feeding or by stroking, respond to the presence/ disappearance of the stockperson in the same way as group-housed lambs to the separation and remixing of their peers (Boivin et al., 2000). In some species, contacts with animals from other species at an early age can lead to socialisation to this species. This has been observed in dogs but also in other species (for review: Scott, 1992).

Price and Wallach (1990) observed that hand rearing of bull calves until 7 months of age in physical isolation from their conspecifics results in aggressiveness towards handlers at 19 months of age. Bull calves that were group-housed never attacked their handler and threatened handlers less than did bulls housed in isolation. An explanation for this could be that bulls living in physical isolation have not learned expression of normal submissive behaviour, whereas group-reared bulls have learned how and when to limit aggressive behaviours through agonistic interactions with their penmates (Price and Wallach, 1990). Alternatively, bulls may recognize humans as a social partner and behave with man as they would with other cattle. Thus, ''socialisation'' to humans may occur in cattle.

2.2 Impact of social isolation on welfare of cattle

Animal management should take into account the demands of species-specific social behaviour. Because cattle are a gregarious species, rearing in isolation can be stressful for them. The degree of isolation can vary from total to partial isolation from conspecifics and can be tactile, auditory, visual or olfactory. Different management strategies have distinct effects on cattle behaviour, production and stress physiology.

2.2.1 Housing effects on behaviour in the home environment

Housing calves alone can affect their gross behaviour. Calves housed in individual crates with the possibility of seeing and touching their neighbours through the front of the crate spend more time licking or nibbling at parts of their crate than calves reared in groups (Veissier et al., 1998a; Blokhuis et al., 2000). This increase in oral activity is even more marked when calves are in total isolation: not only do they spend more time nibbling, but they also spend less time lying down (Waterhouse, 1978; Creel and Albright, 1988; Veissier et al., 1997).

Nearly all veal calves in individual crates (with the possibility of seeing and touching their neighbours through the front of the crate) but less than half of veal calves in groups of four engage in tongue-rolling (Veissier et al., 1998a). However, feeding treatments (milk only and milk plus solid complement) are far more important in other non-nutritive oral behaviours. The most eager tongue-rollers are calves housed in individual crates and fed a liquid-only diet (Veissier et al., 1998a). Albright et al. (1991) observed calves housed in groups of five and calves housed individually in stalls and found no significant differences in oral activities, but no exact information about the degree of isolation was given.

In small crates, the lying patterns of calves are likely to be restricted by the partitions, and in group pens by the other calves (Veissier et al., 1994a). Visually isolated calves in hutches tend to spent more time recumbent than calves reared in groups of six, possibly because they are not disturbed or stimulated by penmates (Warnick et al., 1977). However, Hänninen et al. (2005) found that in pens with concrete floors, duration and frequency of resting on the side are higher for pair-housed than for individually housed calves who could communicate with neighbouring calves through the metal bar walls. Thus, in small groups, penmates do not seem to disturb each others' recumbent behaviour.

Calves in group pens are generally more active in terms of locomotion than calves in individual pens housed with solid pen walls and restricted contact with neighbouring calves through the front of the pen (Jensen et al., 1998). Müller and Schlichting (1991) compared groups of 5 and 10 veal calves and space allowances of 1.0 m^2 and 1.5 m^2. In the uncrowded groups, calves moved more frequently and had more lying bouts, probably because they had fewer difficulties in lying down. However, problems related to recumbency were more related to slatted floors than to space allotment (Müller and Schlichting, 1991). Hence, the increase in movement observed in grouped calves compared with those individually housed was apparently not due to disturbances by other animals but rather by social stimulation. This phenomenon, termed social facilitation or contagious behaviour, refers to the behaviour of a companion releasing a similar performance by the subject (for review: Nicol, 1995). Jensen and Kyhn (2000) found increased locomotor play behaviour (galloping, leaping, jumping and bucking) in calves with increasing available space. Low space allowance reduces calves' locomotor play in both individual- and group-rearing environments (Jensen and Kyhn, 2000).

In conclusion, partially isolated calves display more non-nutritive oral behaviour but less gross activity, such as moving, than group-housed calves. The possibility to moving and to express locomotor play is important for the welfare of calves. Frequent or long-lasting expression of non-nutritive oral behaviour is considered abnormal and may indicate an unsatisfied need for manipulating roughage feed. Thus, according to their behaviour, the welfare of calves kept partially isolated is lower than that of animals housed in groups.

2.2.2 Housing effects on stress physiology and production

Housing calves alone can be stressful for the animals. Calves tethered in individual crates with no possibility of physical contact with neighbouring calves have higher plasma cortisol responses to adenocorticotropic hormone (ACTH) than group-reared calves, and this is considered to be due to chronic stress (Dantzer et al., 1983; Friend et al., 1985). According to Dellmeier et al. (1985), this stress results from calves being highly motivated to interact with other calves. When a calf that has been denied social contact is put in the presence of another calf, it interacts with it more frequently than does a calf that has already had social contacts (i.e. the "damming up" phenomenon, Dellmeier et al., 1985). Corticoids, which are involved in stress responses, have an effect on metabolism by increasing gluconeogenesis at the cost of protein synthesis (Mormède, 1995). Warnick et al. (1977) have shown that visually isolated calves grow slower than calves housed in groups. However, higher basal cortisol levels were found in group-housed calves (housed in groups of 15 or in groups of 4) than in individually housed calves (given the possibility of limited contact with their neighbours through the front of the crate). This might be due to sampling stress as a result of human handling and restraint being greater in group-housed calves (Trunkfield et al., 1991; Veissier et al., 1998a).

Weight gain has been described as being higher in calves reared in groups of six than in either those reared individually in hutches with open partitions or those reared individually in hutches and visually isolated (Warnick et al., 1977). However, Purcell and Arave (1991) report no difference in average daily gain between twin heifer calves in complete visual and spatial isolation and those in groups of 5 or 6. Similarly, Smits and de Wilt (1991) found no differences in daily growth and feed conversion between individually and group-housed veal calves, but the degree of isolation in individual crates was not provided in this study.

Warnick et al. (1977) report that after 10 weeks, when all calves are grouped together, previously group-reared calves start eating concentrate significantly earlier than their previously individually housed but not visually isolated peers, with spatially and visually isolated calves being the slowest group to start eating concentrate. Purcell and Arave (1991) found that group calves spent a longer time eating than their visually and spatially isolated twins. By contrast, Smits and de Wilt (1991) describe no differences in feed conversion between individually (degree of isolation not reported) and group-housed veal calves. Warnick et al. (1977) explains that learning to eat concentrate earlier when housed in a group is the result of exploring and imitating penmates. Dellmeier et al., (1985) suggests that the degree of social facilitation of feeding in a group of animals depends on the early social experience of group members. Group rearing may, however, expose animals to food competition, cause aggressiveness or otherwise alter eating behaviour, especially if there are not enough feeding places or if some feeding places are superior to others. In the study of Bornett et al. (2000), group-housed pigs ate faster, less frequently and for a longer duration than individually housed pigs who were physically isolated from other pigs in adjacent pens. However, this phenomenon is probably limited in calves because of the low level of aggressiveness between them.

Housing female dairy calves for the first 12 weeks of life in groups (either with calves or with cows and calves) or individually (open or closed pen) has no effect on later milk production (Mogensen et al., 1999). Arave et al. (1985) report, however, that female calves reared in physical and visual isolation for the first 10 weeks of life, with or without human handling, later produce significantly more milk than their peers housed in groups or in individual hutches.

In conclusion, isolation of calves results in physiological stress. Social facilitation can increase feeding in group-housed animals as compared with individually housed and visually isolated animals. However, no obvious differences in calves' growth have been detected between the two groups of animals.

2.2.3 Housing effects on reactivity

An open-field test, which consists of exposure to a novel environment, was originally developed to study laboratory rodents' responsiveness to novelty. Recently, this test has also been used among farm animals, including cattle, and is sometimes called ''an arena test'' or ''a novel environment test''. Cattle responses to an unfamiliar arena depend on such factors as previous housing regime, reactions towards the people who transfer them to the arena, duration of exposure to the arena, and whether the animals are presented to the arena alone or in a group. Individual variation in response to unfamiliar arena tests is considerable (Munksgaard and Jensen, 1996). The reactivity of animals can also be assessed when they are confronted with an unusual event such as a water throw test (Veissier et al., 1997) or the sudden opening of an umbrella (Boissy et al., 2001).

Calves housed alone are more disturbed by external events than calves with social contacts. This has been observed both on farms where individually housed calves are visually and acousticly isolated from the farm environment (Webster et al., 1985) and in experimental conditions where calves are physically and visually isolated (arena test: Warnick et al., 1977; water throw test: Veissier et al., 1997). Totally isolated (visual and tactile isolation) calves have a higher plasma concentration of cortisol during handling (Creel and Albright, 1988). Higher activity and reactivity to external events are likely to be energy-consuming. Familiar peers are known to have a calming effect on each other, and in individually housed calves this effect does not exist (Boissy and Le Neindre, 1990; Takeda et al., 2003). Calves housed in total isolation stand more and tend to vocalise and investigate more than individually housed calves who are only partially isolated (Creel and Albright, 1988).

Warnick et al. (1977) observed that group-housed calves vocalise more and are less active when tested alone in an arena than individually housed calves (either visually isolated or not). Similarly, Danzer et al. (1983) noted that previously tethered calves spent less time immobile in an arena test than group-housed calves. Arave et al. (1985) found that group-reared calves did urinate and defecate more often during arena tests than individually housed calves (independent of the degree of isolation), but no differences were present in the frequency of vocalisations.

Calves whose individual pen size was reduced one-quarter the original size before the arena test gallopped and buck-kicked more than control calves who were consistently kept in large pens. The motivation of confined calves to move in the arena is the same if they are confined 4, 2 or 1 week before the test, indicating that internal motivation to move may develop within a few days or even in hours (Jensen, 1999).

In a social test, group-housed calves sniffed, mounted and tended to play-fight more than calves individually housed either in open or closed single pens (Jensen et al., 1999). Individually housed calves with the possibility of only head contact with neighbouring calves in a home pen showed more fear-related behaviours in the arena at three months of age either with another calf or alone than calves housed in groups of four (Jensen et al., 1997). Exploration behaviour of these calves did not differ between the two housing groups. The authors noted that the observed behavioural differences between the two groups in the arena test were no longer present after tethering all calves for three months (Jensen et al., 1997).

Group-housed cattle are normally distressed when separated from their peers (Kilgour, 1975; Purcell and Arave, 1991; Boissy and Le Neindre, 1997) (Table 1). Social separation from conspecifics in heifers results in increased struggling, vocalisation, heart rate and plasma cortisol concentrations. The longer heifers have social contacts before the separation, the greater the distress at separation. Behavioural responses to separation decrease when conspecifics are brought back together (Boissy and Le Neindre, 1997). Social isolation (visual and tactile) changes cows' reactions to a novel environment (Munksgaard and Simonsen, 1996).

In conclusion, individual housing (with different degrees of social isolation) renders calves more reactive to unusual events and more eager to move (when calves are both isolated and confined). However, heifers housed in groups react to separation from their social partners and try to restore contact with them by vocalising.

2.3 Effects of mixing and social instability

Wild or feral groups of cattle are very stable (Bouissou et al., 2001), hence the argument is that farm animals should be housed in stable social environments. The Pig Welfare Advisory Group (DEFRA, Department for Environment, Food and Rural Affairs, UK) advises avoidance of remixing of sows, and the Council of Europe (1988) recommends that 'bulls should not be added to groups already formed.'

Reorganisation of social groups induces stress related behavioural and physiological reactions (Arave and Albright, 1976; Mench et al., 1990). Introduced animals normally have more problems than resident animals (Mench et al., 1990). Mixing of dairy cows results in shortened lying bouts, prolonged standing, reduced time spent eating and decreased milk production (Hasegawa et al., 1997; Phillips and Rind, 2001). Mixing of bulls before slaughter causes behavioural interactions that lower the glycogen content of muscles, resulting in a higher ultimate carcass pH (Warriss et al., 1984). In pigs, fighting and stress responses following mixing, especially during the embryo implantation period, can affect reproduction (for review: Arey and Edwards, 1998). Altogether, the negative effects of mixing on animal welfare and production are considered so great that regrouping should, when possible, be completely avoided (Hasegawa et al., 1997; Bøe and Færevik, 2003).

The effects of grouping on behaviour and production are generally of short duration. In female cattle, fights between new animals are limited to the first hours after regrouping; thereafter, relationships are maintained by threats from the dominant animal and spontaneous avoidance by the subordinate animal (Bouissou, 1974). The mixing of bulls 48 hours before slaughter does not affect ultimate carcass pH (Warriss et al., 1984). However, the milk yield of mixed cows remains lower than that of unmixed cows for 1-2 weeks after mixing (Hasegawa et al., 1997; Phillips and Rind, 2001).

Heifers and calves with prior mixing experiences form more stable relationships, fight less and establish dominance relationships more quickly than their less experienced counterparts (Bouissou, 1975; Veissier et al., 1994b). In addition, mixing experiences seem to improve subsequent social behaviour of cattle: calves that have always been in groups are found at the top of the hierarchy when regrouped with calves that have always been isolated in closed crates (Veissier et al., 1994b). However, calves that have experienced only one grouping dominate calves that have experienced several groupings (Veissier et al., 1994b). Therefore, an optimum level of social experience may exist for cattle.

The effects of mixing or comparable social stressors on hypothalamic-pituitary-adrenal (HPA) axis activity seem to vary in appearance and duration. Friend et al. (1977) report that adrenal response of cows after exposure to regrouping and increased barn density can be detected two days after regrouping. Free stall competition for seven days increases cows' glucocorticoid response to adenocorticotropic hormone (ACTH) challenge (Friend et al., 1979), and cortisol responses of high ranking-heifers to ACTH challenge increase two weeks after mixing (Hasegawa et al., 1997). Mixing beef cows results in increased blood cortisol levels even 84 days after mixing in subordinate alien cows (Mench et al., 1990). Thus, according to its prolonged effects on HPA axis activity, specially on the sensitivity of adrenals to ACTH, social stressors are likely to induce a stress state in cattle.

What happens to the reactions of animals when grouping is repeated? Because grouping causes reactions that are typically found in acute stress situations, repeated grouping might induce chronic intermittent stress (Ladewig, 2000). Chronic stress modifies the functioning of the HPA axis, and modifications can result in hyper- or hypoactivity of the axis or disruptions in axis activity (for review: Tsigos and Chrousos, 2002). Ladewig and Smidt (1989) found that the episodic cortisol secretory pattern (episode frequency, duration and interval) of tethered bulls is altered at the beginning of the restraint period but that it returns to normal after four weeks. Mench et al. (1990), by contrast, reported that cortisol remains higher in subordinate alien cows for 80 days after mixing. Basal cortisol level is perhaps not a proper indicator of physiological state of stress in animals (Ladewig and Smidt, 1989; Klemcke, 1994). Corticotropin-releasing factor (CRF) is a primary regulator of ACTH secretion from the anterior pituitary gland, which regulates the synthesis and secretion of cortisol (for review:

Matteri et al., 2000). Adenocortical reactivity to ACTH stimulation may indicate changes in animals' adaptation to long-term stressors at the brain-pituitary level. Ladewig and Smidt (1989) found that this reactivity is significantly reduced in bulls tethered for five weeks. However, many studies show results contrary to those of Ladewig and Smidt (1989). Tethered bulls deprived of lying down have increased cortisol responses to ACTH after seven weeks of deprivation compared with tethered bulls not deprived of lying down (Munksgaard et al., 1999). Tethering for six weeks enhanced the sensitivity of the adrenal cortex to ACTH in calves (Dantzer et al., 1983) and in pigs (Janssens et al., 1995). In calves, integrated cortisol responses to ACTH challenge are higher in stalled (tied and physically isolated from other calves) and penned (physically isolated from other calves) individuals than in hutch- (tied and physically isolated from other calves) and yard-reared (group-housed) animals (Friend et al., 1985). This suggests that space allowance rather than social contact is essential to avoid chronic stress of calves. Calves that are repeatedly regrouped with others also have increased cortisol responses to ACTH challenge compared with undisturbed calves (Veissier et al., 2001).

There is evidence that chronic stress induced by social instability or isolation alters the reactivity of animals to unexpected events. The emotional reactivity of rats, for instance, is increased by chronic social isolation (Weiss et al., 2004). Tethered sows react less to external events (Broom, 1987), while isolation or repeated mixing leads to higher reactivity in calves (Veissier et al., 1997; Boissy et al., 2001). Chronic stress also induces immune dysregulation, which, in turn can have health implications (Blecha, 2000; Padgett and Glaser, 2003). In addition, repeated stressors can alter the growth of animals, as seen in rats reared in unstable social environments (Mormède et al., 1990).

According to Boissy et al. (2001) and Veissier et al. (2001), repeated regrouping increases calves' reactions to emotionally negative events (exposure to novel or sudden events, to a dog or to restraint) and increases their cortisol responses to ACTH. However, based on their behaviour, calves seem to habituate to repeated mixing, as they do not interact as much with a new partner when they have already been mixed several times (Veissier et al., 2001). Regrouping might have greater effects on older animals than on calves because of social behaviour developing with age (Bouissou, 1977). Regrouping may thus affect heifers' emotional reactivity, physiology and production to a greater extent than it does in calves.

In conclusion, mixing is a stressor for cattle based on animal behaviour, stress physiology and production. Mixing-induced stress might vary for animals according to age.

2.4 On-farm group management of cattle

2.4.1 Human-cattle interactions in group housing

Group-housed cattle are able to express more of their behavioural repertoire than animals in tie stall barns. Group housing may impair human-cattle interactions and handling because group-housed cattle are more difficult to separate from their peers and have more available space to avoid people (Table 1). Poor habituation to humans may result in animals' fear of people, which is one of the major causes of animal handling accidents (Grandin, 1999). Mogensen et al. (1999) found that group-housed calves are more reluctant to approach a human than individually housed calves (Table 1). Trunkfield et al. (1991) reported that calves housed in groups of 15 are more laborious to load into a truck than individually housed calves, and Veissier et al. (1998a) observed that calves housed in groups of four are more difficult to handle for weighing than individually housed ones (who had the possibility of seeing and touching their neighbours through the front of the crate); such calves also responded with higher cortisol levels during weighing. Thus, contact with the stockperson may be less effective for group-housed calves than it is for individually housed calves (Veissier et al, 1998a).

Table 1. Housing effects on human-cattle interactions (adapted from: Raussi, 2003).

	Group housing compared with individual housing	Authors
Approach behaviour to humans	Slower Less frequent	Mogensen et al. (1999), Purcell and Arave (1991)
Separation/restraint	Can be difficult	Boissy and Le Neindre (1997), Veissier et al. (1998a)
Loading	Slower More effort needed	Trunkfield et al. (1991)
Male aggressiveness towards humans	Less aggressiveness	Price and Wallach (1990)
Impact and efficiency of human contact	Minor	Veissier et al. (1998a)

Rearing calves in visual and tactile isolation from conspecifics enables them to better cope with human handling on commercial dairy farms (Purcell and Arave, 1991). Behaviour towards a human after weaning and regrouping was found to differ between a group-reared heifer calf and her twin sister reared in isolation. Group-housed calves do not approach the stockperson as much as do previously isolated calves (Purcell and Arave, 1991) (Table 1). The farmer has a special role in formation of the social environment of an early-weaned calf. Pearce et al. (1989) have, however, suggested that having regular contact with animals reared in groups may lead to reduced effects on their response to humans, compared with animals housed and provided contact individually.

To improve animal welfare in group- or individual-housing settings, the stock-person should spend adequate time with the animals, and food, together with other pleasant stimuli, should be used to reward desired behaviours and provide positive interactions. Krohn et al. (2001) demonstrated that frequent handling of individually housed newborn calves and hand feeding during the first four days of life increases calves' motivation to approach a human. Daily inspection merely by walking among the group of cattle facilitates animals' habituation to humans (Seabrook, 1994). Minimising the role played by humans in negative handling procedures may help to prevent animals from developing a fear of man (Hemsworth and Coleman, 1998).

To conclude, group housing may increase the risk for poorer human-cattle interactions and handling unless positive contacts are provided.

2.4.2 Using social relationships of cattle in management

Learning is fundamental to animal welfare because it enables them to cope with and adapt to changes in their environment. The learning ability of cattle and such learning techniques as habituation and operant conditioning should be optimised in cattle management. In general, animals will learn things that have advantageous consequences for themselves. Habituation of animals to intensive farming conditions, e.g. adapting dairy heifers to the milking parlour before they calve, complements the genetic selection of farm animals more suited to modern farms (Kilgour, 1987). After familiarising some animals in a group to a certain handling procedure, these animals can then act as a model to others. Knowledge of the social identity of animals and the most favourable period for social awareness would be useful in selecting animals to serve as role models (Veissier et al., 1998b).

Living in a group may be assumed to facilitate learning by observation. Veissier (1993) studied whether observational learning could be shown in cattle. When heifers observed their demonstrator peer doing a task, greater attention was focussed on the stimuli involved in the task, which may facilitate learning. Munksgaard et al. (2001) found that cows who observed neighbouring demonstrator cows being treated gently kept a shorter distance to the gentle person. Thus, some social transmission of information may have occurred between neighbouring cows (Munksgaard et al., 2001). Further evidence of faster learning in the presence of a trained partner was found in lambs learning to suck from a teat bucket (Veissier and Stefanova, 1993). Foraging together with experienced social partners may facilitate acceptance of novel foods (Ralphs et al., 1994) and avoidance of harmful foods by naive animals (Ralphs and Olson, 1990).

When learning was studied in visually and physically isolated or group-housed twin heifer calves, the isolated calves achieved the goal in the T-maze test more quickly than their group-housed twins (Purcell and Arave, 1991). Calves had been introduced to the T-maze construction alone. The test may thus have measured more the calves' response to separation and novelty than their learning ability (Purcell and Arave, 1991). This speculation is supported by the observation that heifers seem less afraid of being in a novel arena with their social partners than of being alone (Veissier and Le Neindre, 1992).

In conclusion, abundant possibilities exist to exploit the social and individual behaviour of cattle in management. Species companions in farm animal groups facilitate such learning processes as acceptance of novel foods.

2.5 Summary of literature survey

It is important to understand how the social behaviour of cattle functions in big modern dairy farms where the animals are group housed. Taking social behaviour and relations among animals into account in management will enhance the welfare of animals while simultaneously helping farmers in their work.

Overall, group housing appears to be better for the welfare of calves than housing calves individually in isolation, especially in tactile and visual isolation. Benefits of group housing include a larger available space and an enriched social environment, which can make the animals more resistant to other stressors.

However, some problems in group housing, e.g. poorer human-animal interactions and unfavourable mixing of new animals, do exist.

Cattle reared together develop an affinity towards each other that is expressed by a high frequency of licking, close proximity and synchronised activities. Mixing with other animals induces aggressive encounters that result in the establishment of dominance relations in adult cattle. Repeated mixing may lead to chronic stress.

3 Aims of the study

This study aimed to analyse the importance of species companions on the welfare of young cattle and the extent to which human contacts could compensate for the lack of social partners. Because young cattle are more often subjected to isolation than older cattle, this work was undertaken with calves (I, II). A second aim was to investigate the effect of repeated regroupings on the welfare of cattle. Because social behaviour develops rapidly in puberty, this effect was investigated in heifers (III, IV).

Specific objectives were as follows:

1. to investigate whether pair housing, compared with individual housing, reduces stress reactions in calves or affects their activity or reactivity to external events (I, II);

2. to investigate whether pair housing, compared with individual housing, affects calves' responses to people and handling (I, II);

3. to investigate whether positive contacts with humans or housing in pairs or individually affects calves' preferences for species companion and humans (I);

4. to investigate the effects of additional positive human contact during rearing on the response of calves housed in pairs or individually to people and to handling, i.e. to determine whether the positive effects of handling are equally effective in pair-housed and individually housed calves (I, II);

5. to determine whether repeated regrouping affects activity and social behaviour (aggressive behaviour and affinities) of pair-housed heifers (III, IV);

6. to determine whether heifers habituate to repeated regroupings, i.e. react less strongly to being with new partners, or whether they learn to recognise the dominance value of the new partner, resulting in dominance relations formed more quickly and with less agonistic interactions (III);

7. to analyse the consequences of repeated regrouping on emotional reactivity, stress physiology and production in dairy heifers (IV).

4 Materials and methods

This thesis is based on two experiments. The first was conducted on the Lintupaju farm at MTT, Jokioinen, Finland, and the second on an INRA farm in Marcenat, Cantal, France. Animals in the first experiment were calves and are referred in the text to as calves. Animals in the second experiment were heifers and are referred in the text to as heifers.

The general objectives of these two experiments were to determine the importance of species companionship and human contact on the welfare of calves (Experiment 1; I, II), and to investigate the effect of repeated regroupings on the welfare of heifers (Experiment 2; III, IV).

The study protocols were scrutinised and approved by either the MTT or INRA committee on experimentation in animals. People in charge of rearing the animals or taking samples from them completed a special course on experimental animals approved by either the Finnish Ministry of Agriculture and Forestry or the French Ministry of Agriculture.

4.1 Animals, housing and treatments

A summary of study animals, housing and treatments is presented in Table 2.

4.1.1 Experiment 1

Sixty-four Finnish Ayrshire male calves originating from two MTT farms in Jokioinen were reared in four batches of 16 calves from autumn 1997 to winter 1999. The calves were kept with their dams for three days after birth. Then they were housed in individual pens, where they learned to drink from a teat bucket with human assistance. The stockpersons were instructed to have minimal contact with the calves during this period. When calves were 15.9 ± 1.3 days old, all calves in the batch were moved to the experimental building. In this heated building, lights were on from 06:00 to 18:00 h. The calves were fed milk replacer from teat buckets twice daily, at 07:00 and 15:00 h, and they had free access to concentrates and hay, and water from a nipple. The calves were housed in pens with wooden slatted floors, which were littered with wood shavings once a day without the caretaker entering the pens. For ethical reasons, no calves were in total isolation: wooden partitions between pens were 120 cm high, with slots of 10 cm, through which the calves could see and sniff their neighbours. For each batch of calves, one male and one female stockperson took care of the calves alternately, and no other persons entered the calves' room. No human contact was provided to the calves except during feeding and littering.

At their arrival in the experimental building until the end of the experiment, the calves were allocated to four treatments according to a 2 × 2 factorial design, with two housing and two contact conditions. The age and weight of the calves were balanced between treatments. Regarding housing conditions, half of the calves were individually housed in 1.2 × 1.8 m pens; the remaining calves were pair-housed in 2.4 × 1.8 m pens. Regarding contact conditions, half of the calves in each housing condition received minimal contact with the stockperson, i.e. they saw him/her around feeding and littering but had no physical contact; the remaining calves received additional contact after the meals, five days a week: when the teat bucket was removed 15 min after a milk meal, the stockperson stroked each calf's neck, head and shoulders while speaking in a gentle tone and allowing the calf to suck his/her other hand. This was done for 60 s per calf after the morning meal and for 30 s per calf after the evening meal.

4.1.2 Experiment 2

Thirty-two Holstein-Friesian heifers born in October 2000 served as subjects. They originated from two INRA experimental farms (Marcenat, Cantal, and Les Monts Dore, Puy de Dôme). Soon after birth, the heifers were taken to a calf experimental barn. They were housed in pairs in 1.8 x 2 m pens separated by solid wooden partitions and equipped with straw bedding. The lights were on between 08:00 and 18:00 h. The heifers were fed milk replacer and hay. They were weaned from milk at 12 weeks of age. The pairs of heifers were allocated to two treatments (regrouped vs. control, see below) such that their date of birth weight at birth, and farm of origin were similar between treatment groups. At six months of age, the animals were moved to a second barn without changing the pairs. The pens in which they were accommodated measured 4 x 5 m, and were separated from each other by 2-m-high solid wooden partitions. The floor was covered with straw. The animals were fed hay (10 kg/day/animal) and concentrates (2.5 kg/day/animal) at 08:00 each day, except on those days when food was used in a behavioural test on the same day. When the heifers were 10 months of age, blood sampling and progesterone assays were done twice with a 10-day interval to confirm that heifers had reached puberty. Because some heifers were not cyclic, all were administered a treatment for inducing heat at 10.5 months of age. The heifers received an intramucular injection of 3 mg of Norgestomet (17alpha-acetoxy-11beta-methyl-19-norpreg-4-en-3.20 dione) and 3.8 mg of Oestradiol, and they were implanted with 3 mg of Norgestomet under the skin of the ear for 10 days.

The experimental treatments consisted of variations of the social environment between 11 and 13 months of age. In eight pairs, the heifers were kept in the same pens from the beginning to the end of the treatment period (controls). In the remaining eight pairs, the heifers changed pens and penmates repeatedly from the age of 11 months onwards (regrouped heifers). Each heifer was individually taken out of its pen for weighing. After weighing, control heifers were always returned to their own pen, while regrouped heifers were placed in different pens with new heifers from the same treatment group. This procedure was performed between 14:00 and 16:00 h. It was done twice a week for five weeks, followed by once a week for the next six weeks, for a total of 16 occurrences. At the end of the treatment period, four controls and four regrouped heifers were put together and kept in the same group until adulthood.

Table 2. Summary of animals, housing and treatments used.

	Calves (Experiment 1)	Heifers (Experiment 2)
Number of animals	64	32
Sex	Male	Female
Age (from start to end of treatments)	2–17 weeks	11–13 months
Breed	Finnish Ayrshire	Holstein-Friesian
Housing	Individually or In pairs	In pairs
Human contact	Additional or Minimal	Normal
Treatments	4 Individually housed: Minimal or Additional contact or Pair housed: Minimal or Additional contact	2 Regrouped pairs or Control pairs

4.2 Measures

The spontaneous behaviour of the animals was followed to determine the effect of housing, human contact or regrouping on the activity and social behaviour of animals. Because fearfulness of animals often manifests in situations that are sudden or novel or include cues from predators (for review: Boissy, 1995), we ran behavioural tests to clarify whether animals' reactivity to suddenness, novelty, fear-eliciting situations and unfamiliar animals or humans was affected by housing, human contact or regrouping. The physiological challenge tests (ACTH and CRF challenges) were performed to determine whether treatments had an effect on the functioning of animals' HPA axis at the pituitary and adrenal levels. In addition, production parameters were selected to determine whether housing, human contact and regrouping affects animals' weight gain, feed intake or reproduction. A summary of the measures used is presented in Table 3, and the sequence of the measures is shown in Table 4.

4.2.1 Spontaneous behaviour (I, III)

The first signs of distress in animals are usually behaviour-related. Thus, changes in the time budget or modifications to the daily activity rhythm of an animal may be markers of a change in welfare (Veissier et al., 1989; 2001).

The calves were video recorded for 12 h (from 06:00 to 18:00 h) in one day when they were 6, 10 and 14 weeks old. The heifers were video recorded for 24 h before the 1st regrouping, and two days after the 5th, 12th and 16th regroupings. Behaviours were scan sampled from the videotapes every five minutes. Detailed descriptions of behaviours observed are presented in the original articles, for calves in article I and for heifers in article III. Two classes of behavioural states were observed: posture/activity (heifers and calves) and proximity (heifers). Two postures were distinguished: standing and lying. When standing, the following activities were identified in calves: moving, sniffing or licking an inanimate object, eating or drinking, self-grooming, contact with a neighbouring calf, contact with a penmate (for pair-housed calves) and inactivity; in heifers: lying down, standing immobile, moving, eating, licking salt and drinking. In heifers, proximity included three states: animals in contact with each other, animals not in contact but at a distance smaller than or equal to 1 m and animals at a distance of more than 1 m. In each class, behavioural states were mutually exclusive. The percentage of time spent in a given posture/activity or proximity state, the number of activity changes and the mean duration of posture/activity and proximity bouts were also calculated. Interactions between heifers were recorded as events. The interactions were grouped into agonistic (threat, butt, fight, flight) and non-agonistic (non-agonistic touching, licking, sniffing, sexual) behaviours according to Bouissou et al. (2001). Butting and threatening were classified as efficient if the recipient turned away and non-efficient if the recipient did not turn away. We calculated the latency and frequency of efficient agonistic (efficient threat, efficient butt, fight and flight), non-efficient agonistic (non-efficient threat and non-efficient butt), total agonistic (either efficient or non-efficient), non-agonistic and sexual interactions.

In addition, the behaviour of the heifers was recorded for three hours straight after the 2nd, 7th, 13th and 16th regroupings (III). The activity states distinguished were the same as for observations in 24-h periods (see above). For regrouped heifers, the time to establishment of a dominance relationship was determined as follows: when two heifers were regrouped, aggressive behaviours (butts, threats, fights) were typically displayed by one or both animals, but after a while, this behaviour was expressed by only one heifer (dominant), with the other animal displaying flight behaviour (subordinate).

4.2.2 Behavioural tests (I, II, III, IV)

Behavioural responses to novelty, suddenness and fear-eliciting situations indicate animals' emotional reactivity (Boissy et al., 2001; Désire et al., 2002; 2004), and these responses can be measured either in test settings or in the animals' home environment. In human-animal relationship studies, animals' fear of humans is normally assessed by their approach/avoidance behaviour towards unfamiliar or familiar persons (Hemsworth and Coleman, 1998).

In this study, several behavioural tests were conducted on calves and heifers. In both experiments, the reactivity of animals was assessed through a novel arena test (response to novelty). In Experiment 2, it was further assessed with an umbrella test (response to suddenness) and a dog test (response to a predator cue i.e. fear-eliciting situation). Animals' social reactivity in both experiments was assessed by means of an arena test with an unfamiliar animal. Social reactivity was also assessed in Experiment 1 through calves' preferences for either a person or an animal (preference test), and in Experiment 2 through a feeding competition test. Calves reactivity to humans was assessed via their responses to an unfamiliar person in front of the home pen, in the home pen and in the arena. Calves responses to individual loading onto a truck and short transport were also assessed.

The behaviour of the animals during the arena, dog and social confrontation tests was coded directly into a hand-held computer (Psion Workabout, Psion PLC, UK) using the Observer software package (Noldus, the Netherlands). The preference test and the unfamiliar person in the pen test were recorded by video camera and later encoded using the Observer software package. Reactions to an unfamiliar person in front of the pen, to individual loading onto a truck and short transport, to the umbrella and to the feeding competition test were observed using timers and recorded on paper, and data were later stored in the Microsoft Excel program.

In Experiment 2, the umbrella, the novel arena and the dog tests were repeated to differentiate between responses to the situation per se and to their novelty.

Tests for assessing animals' reactivity to suddenness, novelty and fear-eliciting situations

Umbrella test (IV): The umbrella test for heifers was adapted from Boissy et al. (2001). Three repetitions were run on three consecutive days. The heifers were not given their daily portion of concentrate on the test mornings. A closed umbrella, which could be opened from a remote place, was placed in front of the pen, 1.2 m from the feeding trough. The daily portion of concentrate was then placed in the trough. After the heifers had eaten the concentrate for 10 s, the umbrella was opened suddenly. For each heifer, observers recorded the latency to initially start eating, the reaction to the opening of the umbrella (0 = no reaction, 1 = heifer stops eating but does not move back, 2 = heifer stops eating and takes one step back and 3 = heifer stops eating and takes several steps back) and the latency to resume eating after the umbrella was opened.

Animal alone in an arena (I, IV): The arena in Experiment 1 measured 4 x 4 m, and the one in Experiment 2 measured 5.4 x 7.1 m. The arenas' floors were divided into nine rectangles marked off by white lines either on the floor (Experiment 1) or on the monitor screen (Experiment 2).

In Experiment 1, the mean heart rate of each calf was recorded during each 5 min test by the Polar Vantage NV™ Tester (Polar Electro Oy, Finland) set to record mean heart rate every 5 s. The latency and frequency of behaviours and the duration of behavioural states described below were calculated using the Observer software package (Noldus, the Netherlands).

Calves' reactions to being alone in an unfamiliar arena for 5 min were observed (I). Two classes of behavioural states were recorded: the position of the calf (the square on which the animal had its forelegs) and its activity (running, exploration [sniffing or licking the arena floor, walls or door] or lack of activity). These states were mutually exclusive. The total frequency of entering any square was taken as a comprehensive score of movements in the arena. Buck-kicks were recorded as events, being defined as a calf lifting its bottom and hindlegs and extending at least one hindleg behind its body (Dellmeier et al., 1985).

Heifers' reactions to being alone in an unfamiliar arena for 8 min were observed for three repetitions run on three consecutive days (IV). A bucket, filled with half of the daily concentrate portion of a heifer, was placed in the middle of the arena wall opposite to the door. Two classes of behavioural states were considered: position (the rectangle in which the animal had its forelegs) and activity (exploration [sniffing or licking the arena floor, walls or door], eating, other activity or lack of activity). These states were mutually exclusive. Low vocalisations (mooing with mouth closed), high vocalisations (mooing with mouth open) and defecations were considered events. The time taken to leave the arena was also recorded.

Animal with a dog in the arena (IV): In Experiment 2, we assessed the reactions of heifers towards a dog. A Border collie shepherd and its master participated in this test. Three repetitions were run on three consecutive days. After the heifer had continuously eaten concentrate for 15 s in the same arena as in previous arena tests for heifers, the dog was commanded to go into the arena and sit down to the right of the arena door. After 1 min, the dog was commanded to sit down to the left of the arena door. This procedure was repeated once. The dog was then commanded to exit the arena through the slot. The same behavioural states and events as for the heifers' arena tests alone were recorded. The following behavioural states were added: looking at (face directed towards the dog), stretching the neck towards, sniffing and licking the dog, eating concentrate without looking at the dog and eating concentrate while looking at the dog. Any threats towards the dog were also noted.

Tests for assessing animals' social reactivity

Animal with an unfamiliar animal in the arena (I, III): Calves' reactions to being in the arena with an unfamiliar male calf (not an experimental calf), tethered to the arena, were observed for 5 min (I). The unfamiliar calf was age-matched to the experimental calves, and therefore, it was a different calf in each batch. The same behaviours as in the arena test alone were recorded. The following behavioural states were added: sniffing, licking or touching the unfamiliar calf. Heart rate of each calf was recorded as in other tests performed in the arena (see above).

A social confrontation test was performed to determine whether social responses towards a stranger differed between regrouped and control heifers (III). This test was carried out for all heifers one day before the 16^{th} regrouping in the same arena as tests alone. Two heifers (one control and one regrouped) were placed together in the arena. The first animal introduced was a regrouped heifer in half the tests and a control heifer in the other half. After 4 min, a bucket of concentrate was introduced and the test continued for another 4 min. Thereafter, heifers were returned to their respective home pens. Agonistic interactions, non-agonistic interactions, sexual interactions, and eating behaviour were recorded similarly to the spontaneous behaviour observed over 24 h.

Feeding competition test: The feeding competition test was performed to determine the dominance relationships between eight heifers (four from both treatments) when they were 2.5 years of age (data not reported in the original articles). This test was originally developed by Bouissou (1977). Tests were performed over a two-day period in the same arena as the previous arena tests. The heifers received hay on the test morning but no concentrate. Each animal was tested four times, i.e. once with each animal from the other treatment, for a total of two tests per animal per day. Before each test, a bucketful of concentrate was put in the arena. Two heifers, one for each treatment, were then placed together in the arena for 4 min. Agonistic interactions, non-agonistic interactions, sexual interactions, and eating behaviour were recorded similarly to the spontaneous behaviour observed over 24 h. A heifer was considered dominant if it ate for longer than its opponent.

Preference test (I): The preference of calves for either an unfamiliar calf or an unfamiliar man was assessed in a Y-maze for 2 min. An unfamiliar man stood motionless at the end of one arm and an unfamiliar male calf was attached by a rope at the end of the other arm of the Y-maze. The unfamiliar calf was age-matched to the experimental calves, and therefore, it was a different calf in each batch. The calf to be tested was left in the Y-maze. The location of the calf was coded as a state. Contacts with the unfamiliar calf or man were recorded as events. Contacts included sniffing, licking and touching the unfamiliar calf or man.

Tests for assessing animals' reactivity to humans

Reactions to an unfamiliar person in front of the pen (II): The calves' responses to an unfamiliar person were observed during the morning meal, in a similar way to that described by Lensink et al. (2000a). Ten seconds after a calf had started to drink from the bucket, the unfamiliar person approached from the side and stood motionless facing the calf behind the bucket. The first reaction of the calf to the appearance of this person was noted as 0 = no reaction or 1 = withdrawal. If the calf did not resume drinking within 10 s after the appearance of the person, the test was stopped. If the calf resumed drinking, the unfamiliar person slowly lowered his right hand to try to touch the forehead of the calf and removed his arm slowly after touching. The reaction of the calf was again noted as 0 = no reaction or 1 = withdrawal. The latency to resume drinking after the touch was recorded.

Reactions to an unfamiliar person in the pen (II): An unfamiliar person entered the pen of the calf to be tested and stood motionless for 2 min, near the middle of the side where the feeding barrier was situated. To observe individual responses of calves during the test, calves housed in pairs were separated with a solid plywood plate one hour before being tested. Pairs had been habituated to this separation. The following variables were calculated: latency to contact the person, and frequency and duration of contacts with the person. Contacts included sniffing, licking, nibbling and touching the person.

Arena test with an unfamiliar person (I): Calves' reactions to an unfamiliar person who stood motionless for 5 min in the arena were observed. The same behaviours as for calves' arena tests alone were recorded. The following behavioural states were added: sniffing, licking or touching the unfamiliar person. Heart rate of each calf was recorded as in other tests performed in the arena (see above).

Reactions to individual loading into a truck and short transport (II): Calves' behavioural and physiological reactions were observed during loading into a conventional slaughter truck, during a 30-min journey and during unloading. For each batch, the four calves from the same treatment were loaded individually. The time needed to load a calf, the number of pushes performed during the loading, the time spent running and the numbers of buck-kicks were noted. The effort needed to load the calves was expressed as: (frequency of pushes during loading) / (loading time). The four calves were then transported together for 30 min in the same compartment of the truck. After transport, the calves were unloaded individually from the truck and led back to their pen, over the same distance and through the same alley as during loading. The same variables were noted as during loading. During loading, transport and unloading heart rate of each calf was recorded as in the tests performed in the arena (see above). One minute before loading, 5 min after transport when calves were still in the truck and 2 h after unloading, a blood sample was taken from the jugular vein to determine plasma cortisol concentrations.

4.2.3 Physiological challenges (I, IV)

ACTH challenge for calves and Dexamethasone/ACTH challenge for heifers (I, IV): The ACTH challenge has been used in humans and animals to detect depressive states and chronic stress, respectively. In humans, a higher sensitivity of the adrenals is observed when depression is present (O'Toole et al., 1998). In animals, although controversy exists about the interpretations of this test, most authors report an increase in corticoid release when animals are subjected to chronic stress (Pigs: Janssens et al., 1995; Cattle: Veissier et al., 2001).

The procedures of the ACTH and Dexa/ACTH challenge tests followed those proposed by Veissier and Le Neindre (1988) and Veissier et al. (1999).

For calves (I), one hour after the morning meal, 0.5 IU ACTH (SynacthenND, Novartis Pharma)/kg metabolic weight ($P^{0.75}$ live weight) was injected intravenously into each animal. Blood samples were taken by puncture of the jugular vein before ACTH injection and 30 and 180 min after the injection.

The Dexa/ACTH challenge was performed on all heifers on the 6th and 7th days after the 14th regrouping (IV). A dose of 20 µg/kg body weight (BW) dexamethasone (DectancylND, Roussel, Paris, France) was injected intramuscularly between 17:00 and 18:00 h. The next morning, from 08:00 to 12:00 h, the heifers were tethered before they were intravenously injected with 1 IU/kg BW ACTH (SynacthenND, Novarthis Pharma, Rueil Malmaison, France). Blood samples were taken by jungular venepuncture immediately before the injection of dexamethasone and before and 30, 120 and 180 min after ACTH injection.

CRF challenge for heifers (IV): Besides stimulating ACTH from the pituitary gland, CRF has been shown to directly stimulate glucocorticoid secretion from the adrenal gland in many species (for review: Matteri et al., 2000). The CRF challenge for heifers was run on the 7th day after the 15th regrouping, between 09:00 and 12:00 h, on one heifer randomly chosen from each pair. The two heifers of a pen were tethered before one was intravenously injected with 1 µg/kg BW bovine CRF (Sigma-Aldrich, Saint Quentin-Fallavier, France). Blood samples were taken by jungular venepuncture immediately before the injection and 20, 60 and 90 min after the injection.

Hormonal assays

Blood samples were centrifuged immediately and the plasma stored at -20°C for 24 h and thereafter at -80°C. Plasma ACTH concentrations were determined by radioimmunoassay (RIA, Nichols Institute of Diagnostics, San Juan Capistrano, CA, USA). The detection limit was 1 pg/ml, and the coefficients of variation within and between assays were 2.6% and 8.3% for low (16 pg/ml) and 4% and 6% for high (300 pg/ml) controls, respectively. Plasma levels of cortisol were determined by RIA with an antibody produced by Cognie and Poulin (INRA Tours, France). The detection limit of the assay was 0.02 ng/ml. The within and between assays coefficients of variation were 11% and 22% for low (4 ng/ml) and 7% and 14% for high (32 ng/ml) controls, respectively. The integrated responses to ACTH and to CRF were calculated as the area under the curves (AUC) using the following equation:

$$\sum_{t=1}^{N-1} 1/2(C_t+C_{t+1})\Delta t$$

where N is the total number of blood samples, C is the concentration of ACTH or of cortisol and Δt is the time interval between successive samples.

4.2.4 Production and health (IV)

The calves' growth and feed intake were followed (data not reported in the original articles). Calves were weighed at the beginning (1st week), half way through (8th week), and at the end (17th week) of the experiment. Milk replacer, hay, and concentrate intakes were followed by weighing the remaining amounts five days a week. Calves' feed intake is reported in terms of dry matter.

Weight gains for heifers were calculated from all weighings performed from the 1st to the 16th regrouping. The number of heifers in heat within 50 days of the end of the regrouping procedure (at 14 to 15 months of age) and the times of first insemination and successful insemination were recorded for the 22 heifers to be inseminated right after the end of the regrouping procedure.

In the two experiments, the health of the calves and heifers was checked twice daily, at feeding times, and appropriate medical treatments were given when necessary. The frequency of health problems and the number of days on which an animal received a medical treatment were noted. Health status was expressed as number of diseases and number of days on which an animal received a medical treatment.

Table 3. Summary of measures used in Experiments 1 and 2.

		Experiment 1	Experiment 2
Spontaneous behaviour	Daily behaviour	X (12 h)	X (24 h)
	Behaviour after regrouping		X (3 h)
Behavioural tests	Umbrella		X (3 sessions)
	Arena alone	X	X (3 sessions)
	Arena with a dog		X (3 sessions)
	Arena with an unfamiliar animal	X	X
	Feeding competition		X
	Preference for animal or person	X	
	Unfamiliar person in front of pen	X	
	Unfamiliar person in pen	X	
	Arena with an unfamiliar person	X	
	Loading and transport	X	
Stress physiology	Heart rate	X (Arena tests & Loading and transport)	
	ACTH challenge	X	X (with Dexamethasone)
	CRF challenge		X
Production	Growth	X	X
	Feed intake	X	
	Reproduction		X

Table 4. Sequence of regroupings, weighings and measures in Experiments 1 and 2.

	Experiment 1	Experiment 2
Regrouping		16 times between 11 and 13 months
Weighing	weeks 1, 8 and 17	16 times between 11 and 13 months
Spontaneous behaviour	12 h on weeks 6, 10 and 14	24 h before 1st regrouping, and two days after 5th, 12th and 16th regroupings 3 h right after 2nd, 7th, 13th and 16th regroupings
Umbrella		3 sessions starting from four days after 13th regrouping
Arena alone	week 15	3 sessions starting from four days after 14th regrouping
Arena with a dog		3 sessions starting from three days after 15th regrouping
Arena with an unfamiliar animal	week 15	1 day before 16th regrouping
Feeding competition		2.5 years of age
Preference for animal or person	week 16	
Unfamiliar person in front of pen	week 14	
Unfamiliar person in pen	week 14	
Arena with an unfamiliar person	week 15	
Loading and transport	week 17	
ACTH challenge	week 17	6th to 7th day after 14th regrouping
CRF challenge		7th day after 15th regrouping

4.3 Statistical analyses

Analyses were performed with the SAS statistical package (SAS Institute Inc., USA). Quantitative data were analysed with analyses of variance (ANOVA). Arc sinus transformation was done for all percentages, and log transformation for data resulting from counting (e.g. frequency of behaviour). Specifications for normal distribution and homogeneous variances were checked on the residues. Post hoc comparisons were performed with the ''least-square means'' procedure.

When the observations and tests were run in the home environment (spontaneous behaviour, umbrella test, unfamiliar person in front of and in pen, growth, and feed intake), the pair of animals was considered the observation unit. In Experiment 1, the mean of the pair was taken, and the number of animals (one or two) was used as a weighting factor. In Experiment 2, the pair was taken as a random factor nested in the treatment.

Factors and covariates included in each type of measures for Experiment 1 is presented in Table 5, and for Experiment 2 in Table 6.

For behavioural tests run on individual animals out of their home environment, the animal was considered the observation unit.

The results are expressed as means ± standard errors. The results section will focus on significant results ($P < 0.05$) and tendencies ($P < 0.10$).

Experiment 1: The effects of housing, contact, batch and housing*contact were assessed. For spontaneous behaviours and ACTH challenges, the data were analysed as repeated measures, observations from week 6 and first blood samples being initial values against which other data were compared. For heart rate during the tests in the unfamiliar arena, calves' activity was taken into account by integrating time spent running, frequency of buck-kicking and frequency of line crossing as covariates. For analyses of heart rates at loading, during transport, unloading and after unloading, basal heart rate was included as a co-variable in the model. A t-test for paired data was performed to determine the effect of transport on cortisol levels and to compare behaviour with the unfamiliar calf to that with the unfamiliar person in the preference test. Chi-squares or Fisher exact values were calculated for qualitative data to compare proportion of calves, not averaged in pens (Siegel and Castellan, 1988).

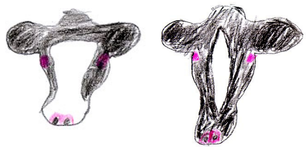
Experiment 2: The effects of treatment (regrouping or control) were assessed. Because pairs of animals changed from one regrouping to the next, the data could not be analysed as repeated data with the pair as a random factor. However, to assess whether heifers learned to form a dominance relationship as the number of regroupings progresses, within-animal comparisons were run for the time necessary to establish the relationship. For behavioural test data, (umbrella, arena and dog tests), the effects of sessions (1, 2 or 3), the treatment and the treatment*session interaction were assessed. Although significant effects of the session number were observed in behavioural tests, they are not commented here unless they interacted with treatments. For social confrontation test performed in the arena with two animals, the order of introduction was considered in the model. For response to dexamethasone, ACTH, or CRF only treatment effects were considered. Chi-squares were calculated to compare proportions.

Table 5. Factors and covariates included in each measure in Experiment 1.

Measure	Housing	Contact	Batch	Time	Inter-action	Covariate	Obser-vation unit	Specifi-cations
Spontaneous behaviour	Fixed factor	Fixed factor	Fixed factor	Fixed factor	Housing* contact	Obser-vations from Week 6	Pair	Repeated measures
Arena alone	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	Activity for heart rate	Animal	-
Arena with an unfamiliar animal	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	Activity for heart rate	Animal	-
Preference for animal or person	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	-	Animal	-
Unfamiliar person in front of pen	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	-	Pair	-
Unfamiliar person in pen	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	-	Pair	-
Arena with an unfamiliar person	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	Activity for heart rate	Animal	-
Loading and transport	Fixed factor	Fixed factor	Fixed factor	-	Housing* contact	Basal heart rate	Animal	-
ACTH challenge	Fixed factor	Fixed factor	Fixed factor	Fixed factor	Housing* contact	First blood samples	Pair	Repeated measures

Table 6. Factors and covariates included in each measure in Experiment 2.

Measure	Treat-ment	Session	Interaction	Covariate	Observation unit	Specifi-cations
Spontaneous behaviour	Fixed factor	-	-	Observa-tions before regroupings	Pair	-
Time to establish a dominance relationship	-	-	-	-	Animal	Regrouping number as a fixed factor
Umbrella	Fixed factor	Fixed factor	Treatment* session	-	Pair	-
Arena alone	Fixed factor	Fixed factor	Treatment* session	-	Animal	-
Arena with a dog	Fixed factor	Fixed factor	Treatment* session	Duration of test	Animal	
Arena with an unfamilar animal	Fixed factor	-	-	-	Animal	Order of introduction added as a fixed factor
Feeding competition	Fixed factor	-	-	-	Animal	-
Dexa/ACTH challenge	Fixed factor	-	-	First blood samples	Animal	-
CRF challenge	Fixed factor	-	-	First blood samples	Animal	-



5 Results

5.1 Spontaneous behaviour (I, III)

Calves

Results for calves' posture and activity over 12 h at 6, 10 and 14 weeks of age are presented in Table 7. The calves were observed lying for 53 (± 2.2) % of daytime hours (from 06:00 to 18:00 h). Pair-housed calves lay down less than individually housed calves. An interaction between housing and contact conditions was found; human contacts decreased lying down in pair-housed calves but increased it in individually housed calves.

Over the course of the experiment, calves spent 0.7 (± 0.2) % of the daytime moving, 10.1 (± 1.4) % sniffing or licking an inanimate object, 22.2 (± 2.0) % eating and drinking, 2.5 (± 0.4) % self-grooming and 2.1 (± 0.7) % in contact with neighbours. In addition, pair-housed calves spent 14.3 (± 3.8) % of their daytime in contact with penmates. Compared with individually housed calves, pair-housed calves were more often in motion and changed activity more frequently. Pair-housed calves were, however, less often in contact with their neighbours. No differences between treatments were observed in sniffing or licking an inanimate object, eating and drinking or self-grooming activities. No effects of contact conditions and no interaction between housing and contact conditions were noted.

Table 7. Effects of housing (individually vs. in pairs) and human contact (minimal vs. additional) on diurnal behaviour of calves. Calves posture and activity in their home pens were scan-sampled every 5 min over 12 h (from 06:00 to 18:00 h) at 6, 10 and 14 weeks of age.

	Housing		Contact		SE	Main effects				Interaction	
	Individual	Pair	Minimal	Additional		Housing		Contact		Housing x Contact	
						F	*P*	F	*P*	F	*P*
Frequency (% scans)											
lying	56.0	50.3	52.6	53.7	2.16	14.9	0.00	0.41	0.53	4.26	0.05
moving	0.57	0.92	0.74	0.74	0.21	7.74	0.01	0.00	0.96	0.02	0.88
contacts with neighbours	2.66	1.02	1.35	2.33	0.75	12.8	0.00	0.94	0.34	0.94	0.34
No. of activity changes between scans	45.2	58.7	51.6	52.3	1.90	57.6	0.00	0.13	0.72	1.37	0.25

Heifers

Activity and proximity of control and regrouped heifers over 24 h before the 1st regrouping and after the 5th, 12th and 16th regroupings are presented in Table 8. Before the 1st regrouping, there were no differences in activity or proximity between heifers from the two treatments. After the 5th regrouping, regrouped heifers tended to be less often in contact with each other and the duration of their contact bouts was shorter than in controls. After the 12th regrouping, regrouped heifers tended to be over 1 m away from each other more often than controls. After the 16th regrouping, regrouped heifers moved and changed activity more often and had shorter activity bouts than controls.

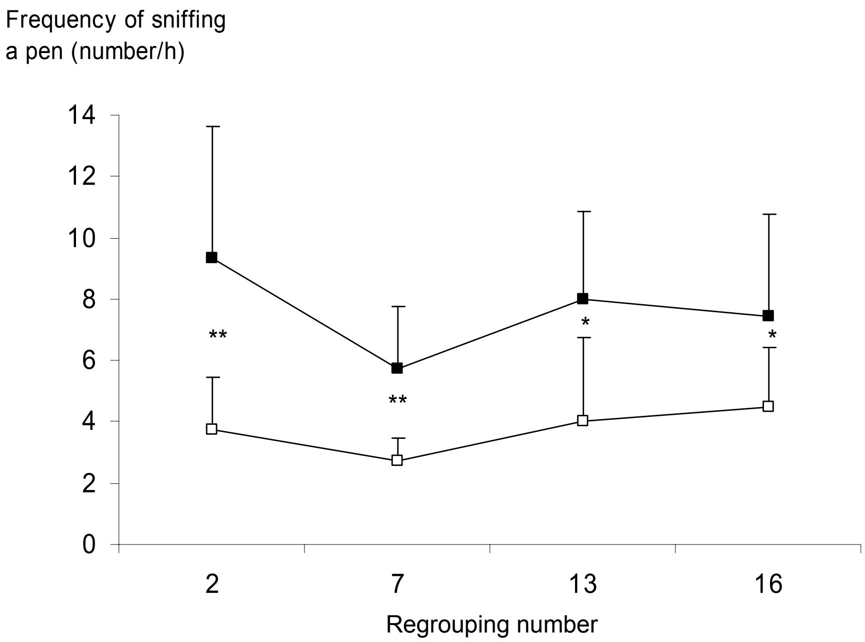
Table 8. Behaviour over 24 h for control and regrouped heifers before the 1st regrouping and two days after the 5th, 12th and 16th regroupings.

	Mean		SE	F	*P*
	Control	Regrouped			
Activity					
before 1st regrouping					
% of scans moving	0.17	0.13	0.00	0.26	NS
Mean duration of activity bout (min)	22.0	20.1	0.10	0.72	NS
Number of activity changes	68.4	72.3	1.45	0.23	NS
after 5th regrouping					
% of scans moving	0.26	0.48	0.00	2.01	NS
Mean duration of activity bout (min)	20.1	19.4	0.10	0.15	NS
Number of activity changes	73.1	75.9	1.92	0.16	NS
after 12th regrouping					
% of scans moving	0.35	0.17	0.00	1.08	NS
Mean duration of activity bout (min)	20.6	18.8	0.09	1.14	NS
Number of activity changes	70.8	77.6	1.65	1.21	NS
after 16th regrouping					
% of scans moving	0.19	0.61	0.00	5,97	0.05
Mean duration of activity bout (min)	23.6	19.5	0.15	5.78	0.05
Number of activity changes	62.8	74.3	2.15	5.31	0.05
Proximity					
before 1st regrouping					
% of scans in contact	1.95	1.60	0.00	0.27	NS
% of scans more than 1 m away	48.3	51.6	0.06	0.12	NS
Mean duration of contact bout (min)	6.30	5.65	0.12	0.36	NS
after 5th regrouping					
% of scans in contact	2.56	1.48	0.00	4.23	0.06
% of scans more than 1 m away	68.0	74.6	0.04	1.85	NS
Mean duration of contact bout (min)	7.90	5.25	0.13	5.07	0.05
after 12th regrouping					
% of scans in contact	1.48	3.77	0.01	1.83	NS
% of scans more than 1 m away	52.3	70.7	0.07	4.34	0.06
Mean duration of contact bout (min)	5.20	6.80	0.14	0.04	NS
after 16th regrouping					
% of scans in contact	2.21	3.39	0.00	1.87	NS
% of scans more than 1 m away	54.8	64.0	0.05	1.53	NS
Mean duration of contact bout (min)	7.20	6.05	0.08	2.01	NS

NS = not significant

In the three hours after the 2nd, 7th, 13th and 16th regroupings, regrouped heifers sniffed their pen more frequently than controls (Figure 2a; $F_{1,\ 14} \geq 5.31$). They also spent more time standing immobile (Figure 2b; $F_{1,\ 14} \geq 9.45$ after the 2nd and 7th regroupings, $F_{1,\ 14} = 3.36$ after the 16th regrouping) and less time lying down (Figure 2c; $F_{1,\ 14} \geq 5.88$ after the 2nd and 7th regroupings, $F_{1,\ 14} = 3.40$ after the 16th regrouping). Regrouped heifers displayed agonistic interactions more rapidly and at a higher frequency than controls who expressed few agonistic interactions (Figure 3a, b; latency of first agonistic interaction: $F_{1,\ 14} \geq 21.0$; frequency of agonistic interactions: $F_{1,\ 14} \geq 10.4$). Agonistic interactions were generally efficient in regrouped heifers and inefficient for controls; after the 2nd, 7th, 13th, and 16th regroupings, 91 ± 2.58, 92 ± 3.24, 86 ± 6.18, and 94 ± 1.49 % of agonistic interactions were efficient in regrouped heifers versus 10 ± 7.28, 6 ± 6.25, 11 ± 6.93, and 33 ± 11.4 % in controls ($F_{1,\ 14} \geq 20.7$, $P < 0.001$). After the 7th regrouping, regrouped heifers had more non-agonistic interactions (Figure 3c; $F_{1,14} = 12.2$) and sexual interactions (20.0 ± 5.32 vs. 1.13 ± 0.43, $F_{1,\ 14} = 9.78$, $P < 0.01$) than controls, a tendency that continued up to the 16th regrouping (17.6 ± 3.38 vs. 7.50 ± 1.65, $F_{1,\ 14} = 4.10$, $P = 0.06$).

2a



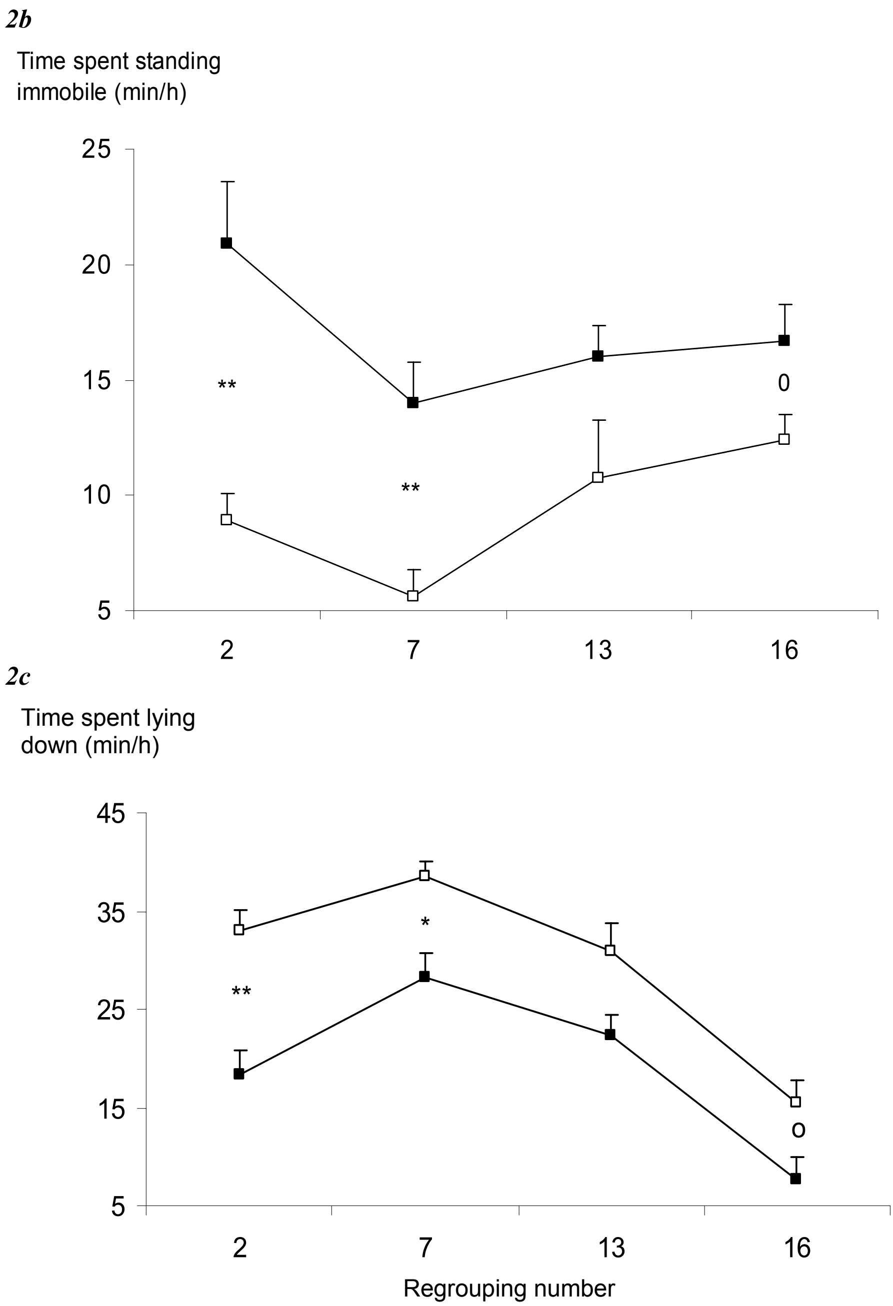
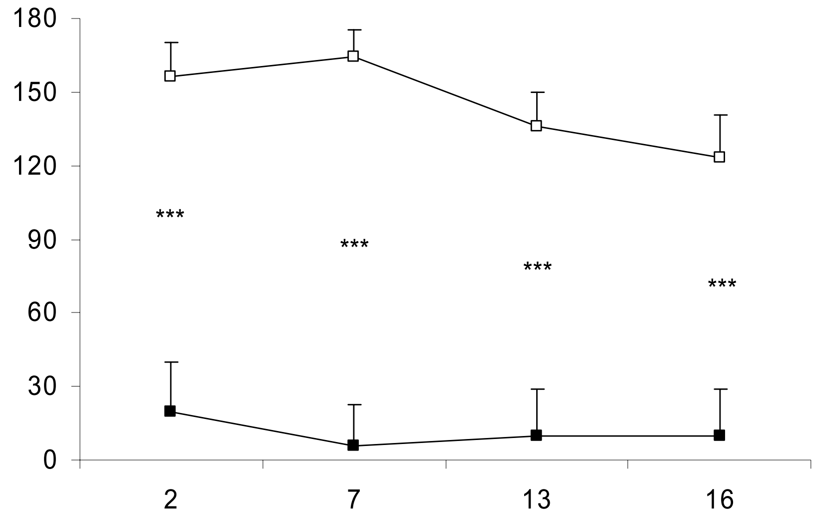


Figure 2: Activity of heifers during the three hours following a regrouping. Regrouped heifers (n = 8 pairs, ■) are compared with controls (n = 8 pairs, □). Figure 2a, frequency of sniffing a pen; Figure 2b, time spent standing immobile; Figure 2c, time spent lying down. ANOVAs were run at each time point to compare treatments; ^{o}, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. F values are given in the text.

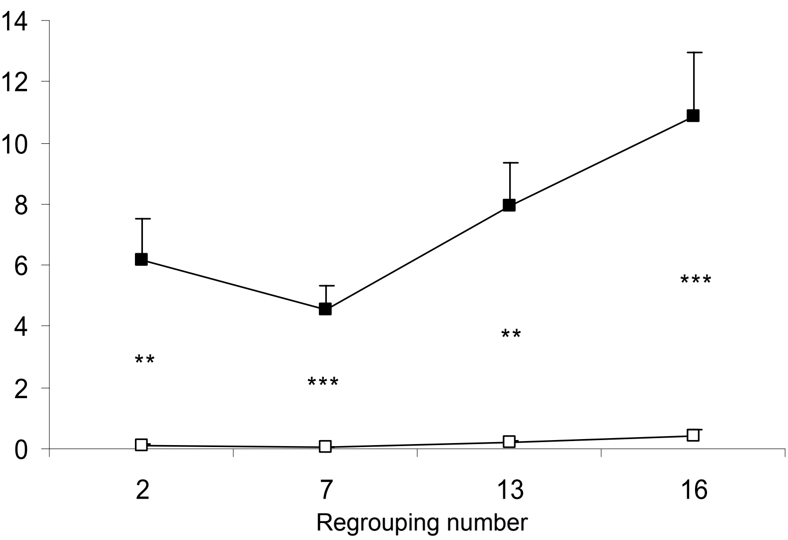
3a

Latency of first agonistic interaction (min)



3b

Frequency of agonistic interactions (number/h)



3c

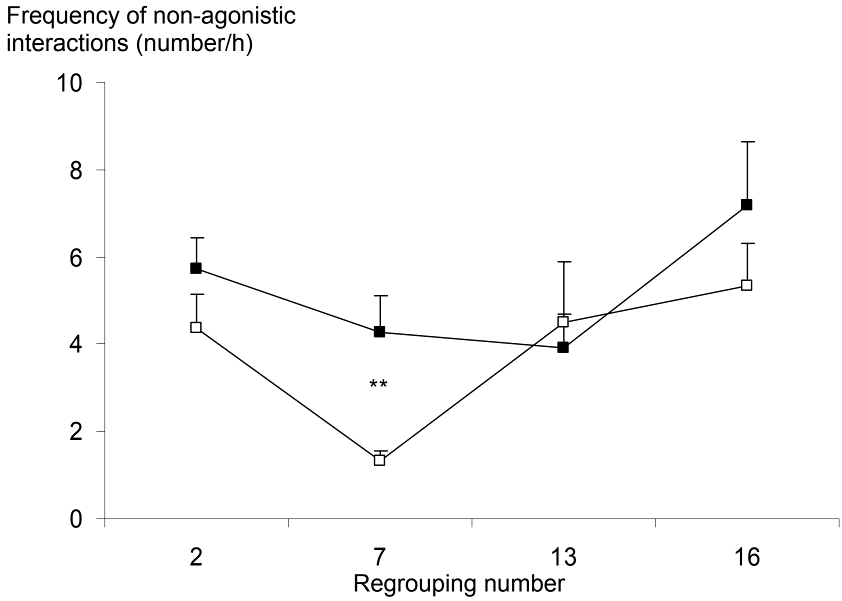


Figure 3: Agonistic and non-agonistic interactions of heifers during the three hours following a regrouping. Regrouped heifers (n = 8 pairs, ■) are compared with controls (n = 8 pairs, □). Figure 3a, latency of first agonistic interaction; Figure 3b, frequency of agonistic interactions; Figure 3c, frequency of non-agonistic interactions. ANOVAs were run at each time point to compare treatments; *, P < 0.05; **, P < 0.01; ***, P < 0.001. F values are given in the text.

The time taken by regrouped heifers to establish a dominance relationship was significantly lower at the 7th regrouping than at the 2nd or 16th regrouping ($F_{1, 15} \geq 7.25$, $P < 0.05$), with a tendency towards a significant difference between the 7th and 13th regrouping ($F_{1, 15} = 3.73$, $P = 0.07$).

In summary, spontaneous behaviour results show that when calves and heifers are undisturbed they exchange few social interactions and these are more non-agonistic than agonistic. Individually housed young cattle are less active than animals housed with stable pairs, who in turn are less active than animals housed in unstable pairs. The opposite is found with regard to recumbency; heifers housed in stable pairs lie down for longer than heifers kept with unstable pairs. Regrouping of pair-housed heifers increases agonistic, non-agonistic and sexual interactions; however, the increase is more marked for agonistic interactions.

5.2 Behavioural tests (I, II, III, IV)

5.2.1 Tests for measuring animals' reactivity to suddenness, novelty and fear-eliciting situations

a) Umbrella test (IV)

During the tests control heifers tended to take longer to start eating in front of the closed umbrella than regrouped heifers (3.80 s for controls vs. 3.23 s for regrouped animals, SE = 0.31, F = 3.40, P = 0.09). Controls also had stronger reactions to the opening of the umbrella than regrouped heifers (reaction 0: 16 vs. 22 heifers, reaction 1: 10 vs. 12, reaction 2: 10 vs. 10, and reaction 3: 12 vs. 2, Chi-square = 8.23, $P < 0.05$). Finally, compared with regrouped animals, controls took longer to resume eating after opening of the umbrella, but this difference was not significant (2.73 s for controls vs. 1.67 s for regrouped, SE = 0.70, F = 2.05, P = 0.17).

b) Animal alone in an arena

Calves (I)

When calves were tested alone in the arena, the ones housed in pairs tended to spend less time running and the distance they covered (either walking or running) was shorter than individually housed calves (Table 9a). No effect of contact conditions and no interaction between contact and housing conditions were found. No differences in heart rate were present between treatments (Figure 4, Test 1).

Table 9. Effects of housing (individually vs. in pairs) and previous human contact (minimal vs. additional) on behaviour of calves in an unfamiliar arena. Calves' behaviour in a 4 x 4 m arena was recorded for 5 min either a) alone, b) with an unfamiliar calf or c) with an unfamiliar person.

	Housing		Contact		SE	Main effects			
						Housing		Contact	
	Indivi-dual	Pair	Mini-mal	Addi-tional		F	*P*	F	*P*
9a									
Arena alone									
Frequency of line crossing	63	42	50	55	6.8	3.7	0.06	0.20	0.68
Time spent running (s)	22	3	9	15	5.2	3.8	0.06	1.25	0.27
Frequency of buck-kicks	4	3	4	3	0.8	0.5	0.47	0.13	0.72
9b									
Arena with unfamiliar animal									
Frequency of line crossing	47	28	37	37	5.1	4.6	0.04	0.01	0.92
Time spent running (s)	9	1	4	6	3.6	1.3	0.26	0.00	0.97
Frequency of buck-kicks	7	3	6	4	1.2	4.1	0.05	0.49	0.49
Latency to contact with calf (s)	66	53	50	69	11.0	0.10	0.75	0.42	0.52
Frequency of contacts with calf	12	14	12	14	0.91	0.85	0.36	2.58	0.12
Time spent in square with calf (s)	94	100	92	101	9.60	0.17	0.68	0.30	0.57
9c									
Arena with unfamiliar person									
Latency to contact with man (s)	70	75	88	57	14.2	0.06	0.81	1.89	0.18
Frequency of contacts with man	9	9	7	12	1.08	0.03	0.86	7.39	0.01
Time spent in square with man (s)	101	83	75	109	11.6	0.87	0.36	3.23	0.08

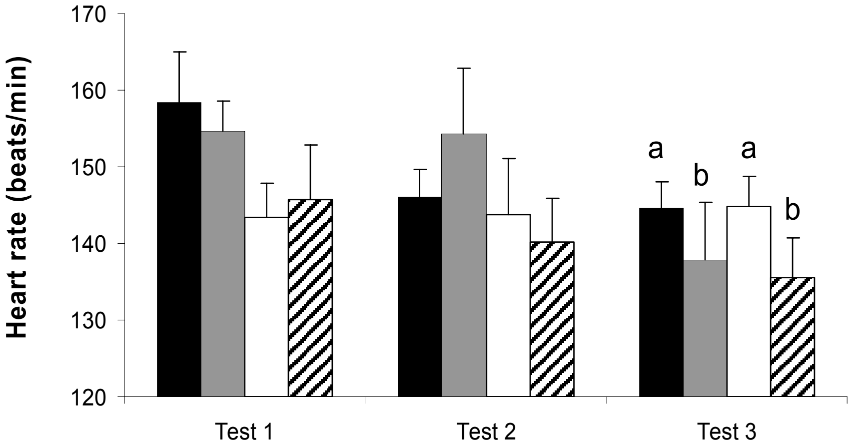


Figure 4. Mean heart rate in Test 1 (arena test alone), Test 2 (arena test with an unfamiliar animal) and Test 3 (arena test with an unfamiliar person) for: individually housed, minimal contact = ■, individually housed, additional contact = ■, pair-housed, minimal contact = □ and pair-housed, additional contact = ▨ calves. Treatments with no common letter differ significantly (P < 0.05).

Heifers (IV)

Results from treatment effects on heifers' behaviour in the arena test are presented in Table 10a. The regrouped heifers started eating more quickly than the controls, this being more marked in Session 1 (F (treatment x session) = 8.71, P < 0.001). Regrouped heifers also had more eating bouts in Session 1 than in subsequent sessions (number of eating bouts: 13.2 in Session 1 vs. 9.4 in Sessions 2 and 3), whereas controls had the same numbers of eating bouts (11.1) over the sessions. Regrouped heifers had more eating bouts than did controls in Session 1, but fewer bouts in Session 2 (F (treatment x session) = 4.23, P < 0.05). The regrouped heifers went out of the arena more quickly than the controls.

Table 10. Results from the arena tests a) alone and b) with a dog of 16 re-grouped (16 changes of partner and pen) and 16 control heifers (maintained in stable pairs and same pen).

	Mean		SE	F	*P*
	Regrouped	Control			
10a **Arena alone**					
Frequency of entering rectangle	35.9	33.5	5.14	0.17	0.68
Time spent next to bucket (s)*	362	354	19.1	0.14	0.71
Time spent next to door (s)*	25.4	27.6	5.34	0.13	0.72
Time spent sniffing arena (s)	67.7	72.4	7.47	0.33	0.57
Frequency of low vocalisations	3.52	3.54	0.91	0.00	0.98
Frequency of high vocalisations	2.13	1.69	0.63	0.38	0.54
Frequency of defecations	0.15	0.27	0.09	1.66	0.21
Latency to eat (s)	8.84	26.9	4.90	10.8	< 0.01
Number of eating bouts	10.7	11.1	1.31	0.10	0.76
Time spent eating (s)	295	277	21.6	0.55	0.46
Latency to go out of arena (s)	13.4	27.1	3.87	9.93	< 0.01
10b **Arena with dog**					
Frequency of entering rectangle	7.52	13.3	2.06	6.26	< 0.05
Time spent next to bucket (s)*	218	192	12.8	3.41	0.07
Time spent next to door (s)*	7.49	15.4	4.06	3.14	0.09
Time spent next to dog (s)*	6.35	11.0	2.98	1.95	0.17
Time spent sniffing arena (s)	6.35	7.42	1.78	0.29	0.59
Frequency of low vocalisations	0.02	0.21	0.09	3.30	0.08
Frequency of high vocalisations	0.00	0.02	0.02	0.96	0.33
Latency to eat with dog (s)	5.84	9.23	4.88	0.39	0.54
Number of eating bouts	6.81	6.96	0.74	0.03	0.86
Time spent eating (s)					
while seeing dog	102	109	16.5	0.16	0.69
while not seeing dog	75.1	37.5	17.2	3.83	0.06
Latency to look at dog (s)	25.2	8.92	8.09	3.32	0.08
Frequency of looking at dog	7.33	9.54	1.04	3.63	0.07
Time spent looking at dog (s)	34.6	48.0	7.03	2.90	0.10
Latency to sniff dog (s)	163	137	23.5	1.03	0.32
Frequency of sniffing dog	1.92	1.81	0.48	0.04	0.85
Time spent sniffing dog (s)	12.6	12.0	3.45	0.02	0.88
Frequency of threatening dog	0.10	0.10	0.06	0.00	0.99
Latency to go out of arena (s)	10.4	9.56	1.93	0.15	0.07

* in the rectangle(s) with the bucket, door or dog, respectively.

c) Animal with a dog in the arena (IV)

Treatment effects on heifers exposed to the arena with a dog are presented in Table 10b. Compared with the controls, the regrouped heifers moved around the arena less. They also tended to spend more time near the bucket, more time eating without looking at the dog and less time near the door, and to have low vocalisations less often. They tended to look at the dog later, less frequently and for a shorter time. The frequency of threats to the dog decreased between Session 1 and later sessions in the regrouped heifers and between Sessions 2 and 3 in the controls; the regrouped animals threatened the dog more often than did the controls in Session 1 (0.31 vs. 0.06), whereas the opposite was true in Session 2 (0 vs. 0.25), and threats were absent in Session 3 (F (treatment x session) = 4.05, $P < 0.05$).

In summary, pair-housed calves are less active when alone in a novel arena than individually housed calves. Regrouped heifers, as compared with controls, react less to suddenness and fear-eliciting situations. However, their reactions are faster (latency to react is shorter) and also subside faster than those of controls.

5.2.2 Tests for assessing animals' social reactivity

a) Animal with an unfamiliar animal in the arena

Calves (I)

When the calves were in the presence of the unfamiliar calf in the arena, those who had been housed in pairs moved from one square to another and buck-kicked less often than did their individually housed counterparts (Table 9b). No effect of contact conditions and no interaction between housing and contact conditions were found. No differences in heart rate were present between treatments (Figure 4, Test 2).

Heifers (III)

During the social confrontation test, when two heifers, one from both treatments, were introduced in the arena, heifers had an average of 11.7 (± 1.12) agonistic interactions, 2.75 (± 0.44) non-agonistic interactions, 3.25 (± 0.96) sexual interactions, and they ate for 1.54 (± 0.17) min, with no significant differences between the two treatments.

b) Feeding competition test

During the feeding competition test eating time tended to be longer in regrouped heifers than in controls (149 ± 14.9 s vs. 98 ± 17.5 s in regrouped and control heifers, respectively; SE = 13.1, F = 5.05, P = 0.07). Regrouped heifers dominated their opponent in 10 of the 16 tests, with no significant difference observed between treatments. Animals had on average 6.28 (± 0.83) agonistic interactions and 13.1 (± 1.07) non-agonistic interactions, with no significant differences seen between treatments.

c) Preference test (I)

The preference test was interrupted for two calves that had received minimal contact, one in each housing condition, because they jumped out of the construction. Data for these calves were therefore not used in the analyses. When released in the Y-maze, most pair-housed calves went to the arm leading to the calf before the one leading to the man and made their first physical contact with the calf, whereas calves housed individually went equally to the two arms and made their first contact equally with the calf and the man (Table 11a). During the test pair-housed calves spent more time near the calf than near the man (paired t-test = 4.5, $P < 0.001$) and made contacts with the calf more frequently than with the man (paired t-test = 4.2, $P < 0.001$). Calves housed individually spent the same amount of time near the calf as near the man, and made the same number of contacts with the calf as with the man (paired t-test = 0.86 and 0.03, $P > 0.10$). Compared with their individually housed counterparts, pair-housed calves spent less time near the man and more time in the middle zone, and they made less contact with the man and more contacts with the calf (Table 11a). No effects of contact conditions and no interaction between housing and contact conditions were found.

In summary, neither individual housing nor repeated regrouping affects the reactions of young cattle to an unfamiliar species companion in the arena. Pair-housed calves show a clear preference for a species companion regardless of their handling treatment.

Table 11. Effects of housing (individually vs. in pairs) and previous human contact (minimal vs. additional) on the behaviour of calves in a) the preference test and b) during loading and unloading into a truck. The behaviour of calves was observed for 2 min in a Y-maze, with one arm leading to an unfamiliar calf and the other to an unfamiliar man. Calves were individually loaded into and out of the truck.

	Housing		Contact		SE	Main effects			
						Housing		Contact	
	Individual	Pair	Minimal	Additional		F	*P*	F	*P*
11a									
Preference test									
Time spent in arm with calf (s)	58.1	71.6	63.5	66.2	6.73	1.42	0.24	0.04	0.84
Time spent in arm with man (s)	44.4	15.3	29.1	30.6	6.80	6.78	0.01	0.04	0.84
Time spent in middle zone (s)	17.5	31.7	27.4	21.8	3.50	8.88	0.00	0.67	0.42
Frequency of contacts with calf	2.70	4.40	3.80	3.40	0.47	4.98	0.03	0.26	0.61
Frequency of contacts with man	2.70	0.94	1.50	2.10	0.46	5.37	0.03	0.79	0.38
						X^2	*P*	X^2	*P*
No. of animals going first to arm with calf	15	22	17	20					
No. of animals going first to arm with man	16	6	12	10		8.90	0.01	0.70	0.71
No. of animals staying in middle zone	0	3	1	2					
No. of animals making first contact with calf	15	23	17	21					
No. of animals making first contact with man	15	5	11	9		7.70	0.02	0.60	0.76
No. of animals making no contact	1	3	2	2					

	Housing		Contact		SE	Main effects			
	Indivi-dual	Pair	Minimal	Additional		F	*P*	F	P
11b									
Loading and unloading									
Loading in to the truck									
Time to load (s)	40.1	56.4	47.4	49.5	3.10	11.2	< 0.01	0.02	0.88
Number of pushes needed	3.10	6.00	5.40	3.70	0.40	23.9	< 0.01	8.67	< 0.01
Effort to load[a]	7.50	10.8	11.4	6.85	0.70	15.0	<0.01	21.6	<0.01
Unloading from the truck									
Time to unload (s)	37.7	55.6	50.3	43.0	3.50	8.01	< 0.01	1.64	0.21
Number of pushes needed	2.25	4.85	4.55	2.55	0.40	7.81	< 0.01	3.77	0.06
Effort to unload[a]	6.10	8.00	8.20	5.90	0.60	3.28	0.08	2.16	0.15

[a]Effort to load = (frequency of pushes during loading) / (loading time); effort to unload = (frequency of pushes during unloading) / (unloading time).

5.2.3 Tests for assessing animals' reactivity to humans

a) Reactions to an unfamiliar person in front of the pen (II)

At the approach of an unfamiliar person in front of the pen during the calves' milk meal, no significant effect of housing or contact treatment was found on the number of calves that showed a withdrawal response. All calves resumed drinking within 10 s of appearance of the person. When the unfamiliar person touched the calves, calves that had received additional contact showed less withdrawal responses and had a shorter latency to resume drinking than calves that had received minimal contact (Table 12a).

Table 12. Effects of housing (individually vs. in pairs) and human contact (minimal vs. additional) on behaviour of calves towards two unfamiliar persons either a) approaching them in front of their pen or b) standing motionless in their pen for 2 min.

	Housing		Contact		SE	Main effects			
	Individual	Pair	Minimal	Additional		Housing		Contact	
						F	*P*	F	*P*
12a **Unfamiliar person in front of pen**									
Withdrawal at approach (no. of calves)[a]	4	4	4	4	-	-	1	-	1
Withdrawal at touch (no. of calves)[a]	19	14	22	11	-	-	0.32	-	0.01
Duration of withdrawal (s)	1.8	1.1	1.9	1.0	0.3	2.24	0.14	4.39	0.04
12b **Unfamiliar person in pen**									
Latency to first interaction (s)	10.3	34.7	23.5	21.4	3.3	13.7	<0.01	0.02	0.88
Total time interacting (s)	84.5	72.4	70.5	86.5	3.6	3.45	0.07	6.04	0.02
Frequency of interactions	13.6	10.3	11.3	12.6	0.7	8.25	<0.01	1.33	0.25

[a] Fisher exact test on qualitative data not averaged in pens.

b) Reactions to an unfamiliar person in the pen (II)

After entrance of the unfamiliar person into the pen, calves housed individually interacted faster and more frequently than their pair-housed counterparts (Table 12b). Additional contact calves interacted longer with the person than did calves with minimal contact, while pair-housed calves tended to interact for less time with the person than did individually housed calves. No interaction between housing and contact condition was found.

c) Arena test with an unfamiliar person (I)

When the calves were exposed to an unfamiliar person in the arena, calves that had received additional contact more frequently made contact with the person than those that had received minimal contact and also tended to spend more time on the square where the person stood (Table 9c). No housing effect and no interaction between housing and contact conditions were found. Lower heart rates were observed in calves that had received additional contacts than in their minimal contact peers ($F_{1, 44}$ (contact) = 5.6, $P < 0.05$) (Figure 4, Test 3).

d) Reactions to individual loading into a truck and short transport (II)

Significantly more pushes and time were needed to load and unload pair-housed calves than individually housed calves, and less effort was needed to load calves that had received additional contact than to load those that had received minimal contact (Table 11b). A tendency ($F_{7, 40} = 3.58$, $P = 0.07$) for an interaction effect was present for effort to unload, indicating that for pair-housed calves without human contact more effort was needed to unload them from the truck and to deliver them to the calves' room than for any other calves. No significant differences were found in the frequency of buck-kicking and time spent running during loading or unloading. No treatment differences were observed in heart rate of calves during the 10 min preceding loading and the 5 min after loading (Figure 5). During loading calves that had received additional contact had a significantly lower heart rate ($F_{7, 40} = 6.74$, $P = 0.01$) than calves that had received minimal contact. A significant interaction between housing and contact condition was found ($F_{7, 40} = 4.17$, $P = 0.05$) during loading, indicating that individually housed calves with additional contact had a lower heart rate than any other calves. Furthermore, calves with additional contact tended to have a lower heart rate at unloading ($F_{7, 40} = 2.95$, $P = 0.09$) than calves that had received minimal contact. During the 30-min transport calves that were housed in pairs had a significantly lower heart rate than calves that were housed individually ($F_{7, 40} = 6.14$, $P = 0.02$). The blood cortisol level increased significantly after transport (Δ(immediately after transport – before loading) = 14.2 ± 2.0, $t = 7.2$, $P < 0.01$) and returned to baseline within 2 h of transport (Δ(2 h after unloading – before loading) = -1.3 ± 0.8, $t = 1.5$, $P > 0.05$) (Figure 6). No treatment effects or interactions were found in cortisol response to loading and transport.

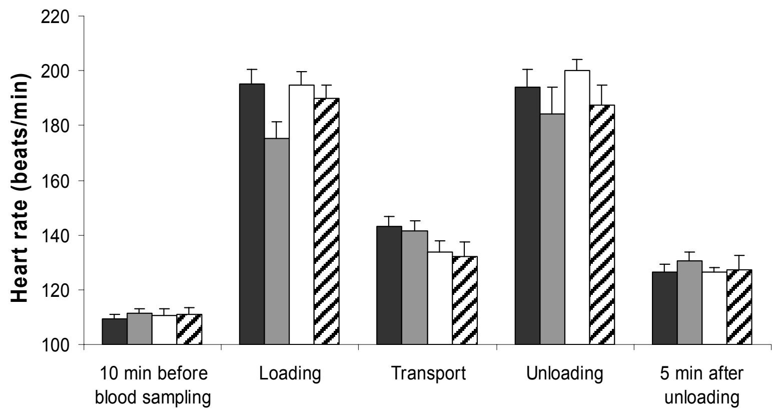


Figure 5. Mean heart rate during 10 min before loading, loading, 30-min transport in the truck, unloading and 10 min after unloading for: individually housed, minimal contact = ■, individually housed, additional contact = ■, pair- housed, minimal contact = □ and pair-housed, additional contact = ▨ calves.

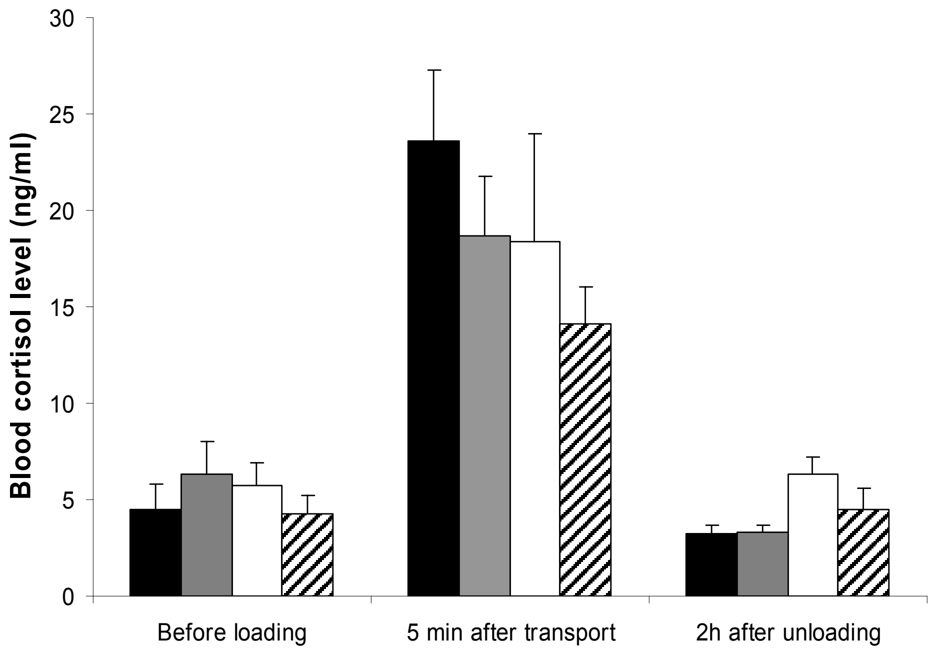


Figure 6. Blood cortisol level (ng/ml) before loading, after 30-min transport in a truck and 2 h after unloading for: individually housed, minimal contact = ■, individually housed, additional contact = ■, pair-housed, minimal contact = □ and pair-housed, additional contact = ▨ calves.

In summary, individually housed calves interact faster and more often with the person entering their pen than pair-housed calves. Pair-housed calves are more difficult to load and unload than individually housed calves, but they have lower heart rates during transport. Calves that have received additional human contact withdraw less when approached in front of their pen, interact longer with the person in their pen, and more often make contact with the person in the arena than calves that have received minimal human contact. Additional contact calves also have lower heart rates with an unfamiliar person in the arena than their minimal contact counterparts.

5.3 Physiological challenges (I, IV)

a) ACTH challenge for calves and Dexamethasone/ACTH challenge for heifers (I, IV)

In calves, plasma cortisol increased after the injection of ACTH. Changes in calves blood cortisol levels over time after the administration of ACTH varied according to housing conditions ($F_{2, 80}$ (time*housing) = 4.3, $P < 0.05$), with a lower increase 30 min after ACTH administration in pair-housed calves ($F_{1, 40}$ = 4.5, $P < 0.05$) (Figure 7). No contact effect and no interaction between contact and housing conditions were found.

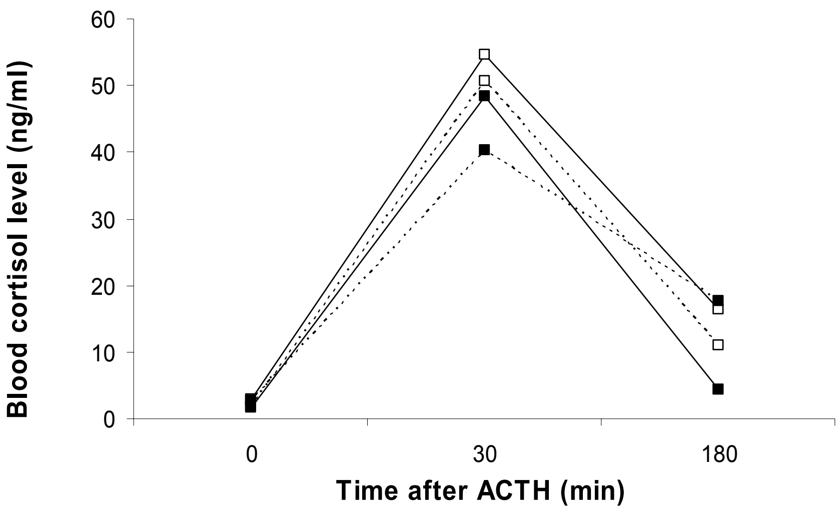


Figure 7. Cortisol response to exogenous ACTH in calves reared in individual pens (□) or pair-housed (■) and receiving minimal human contact (----) or additional human contact (——). Between- and within-subject standard errors were 2.07 and 1.10, respectively.

In heifers, no differences in cortisol responses to the dexamethasone and ACTH challenge were observed between treatments (Table 13a).

b) CRH challenge for heifers (IV)

In the CRF challenge, the regrouped heifers had ACTH responses similar to those of the controls, but lower cortisol responses (Table 13b).

Table 13. Results from a) Dexa/ACTH and b) CRF challenges of 16 regrouped (16 changes of partner and pen) and 16 control heifers (maintained in stable pairs and in same pen).

	Mean		SE	F/X^2	*P*
	Regrouped	Control			
13a					
Dexa/ACTH challenge	n = 16	n = 16			
Plasma cortisol before dexamethasone (ng/ml)	2.88	4.03	0.50	1.41	0.25
Cortisol after dexamethasone (ng/ml)[a]	0.67	0.78	0.12	0.23	0.64
AUC[b] of cortisol (ng x min/ml)	8255	8608	254	0.52	0.48
13b					
CRF challenge	n = 8	n = 8			
AUC of cortisol (ng x min/ml)	1637	2344	170	4.96	< 0.05
AUC of ACTH (pg x min/ml)	10076	11355	1322	0.27	0.61

[a]blood samples taken 15 h after IM dexamethasone injection
[b]area under the curve

In summary, pair-housed calves have lower cortisol increases to ACTH challenge than individually housed calves, and regrouped heifers have lower cortisol responses to CRF challenge than control heifers.

5.4 Production and health (IV)

No differences between treatments were observed in calves (Table 14) or heifers (Table 15) in growth, or in heifers in reproduction (Table 15). However, calves that had received minimal contact tended to eat more hay than calves that had received additional contact (Table 14).

Table 14. Effects of housing (individually vs. in pairs) and previous human contact (minimal vs. additional) on calves' feed intake and growth.

	Housing		Contact		SE	Main effects			
	Individual	Pair	Minimal	Additional		Housing		Contact	
						F	*P*	F	*P*
Hay intake (g/DM/day)	404	451	464	390	0.03	1.22	0.27	3.03	0.09
Concentrate intake (g/DM/day)	1302	1270	1276	1296	0.05	0.16	0.69	0.06	0.80
Milk powder intake (g/DM/day)	476	476	476	476	0.00	0.60	0.44	0.32	0.57
Growth (g/day)	987	989	985	992	0.02	0.01	0.93	0.06	0.81

Table 15. Growth and reproduction of 16 regrouped (16 changes of partner and pen) and 16 control heifers (maintained in stable pairs and in same pen).

	Mean		SE	F/X^2	*P*
	Regrouped	Control			
Growth (g/day)[a]	699	645	26.2	1.15	0.29
Days before first insemination[b]	20.8	29.1	4.17	1.08	0.31
Days before successful insemination[b]	292	119	85.5	1.13	0.30
No. of heifers in heat within 50 days[b,c]	8	7		0.21	0.65

[a]Calculated from 11 to 14 months of age

[b]Calculated from 14 months of age

[c]Fisher exact probabilities

Diarrhoea and navel inflammation were the most common diseases in Experiment 1. During the experiment calves were diseased on average 2.68 (± 0.43) days and had 0.88 (± 0.23) days of medical treatment. No effect of housing or contact conditions and no interaction between housing and contact conditions were found for the number of disease days or for the number of medical treatment days. One regrouped heifer had lameness during the experiment. No other heifers had health problems.

In summary, no growth, health or reproduction effects were detected deriving from individual or pair housing or repeated regrouping.

6 Discussion

Calves housed in pairs differed from calves housed individually in diurnal behaviour, responses to conspecifics and humans and stress responses, but not in growth or health. Pair-housed calves were more active in their home pen but less active alone in a novel arena than individually housed calves, and they clearly preferred an unfamiliar calf to an unfamiliar human. Pair-housed calves showed a lower cortisol response to ACTH challenge than individually housed calves. Additional contact made all calves more eager to approach humans and facilitated handling. Pair-housed but repeatedly regrouped heifers were more aggressive after all observed regoupings and tended to keep a larger distance between each other after 5th and 12th regoupings than heifers kept in unchanged pairs. Heifers that had been repeatedly regrouped reacted less than controls during the behavioural tests: they had milder responses to sudden events and started eating more quickly and tended to eat for longer without looking at the dog in behavioural tests. They also had lower cortisol responses to exogenous CRF, but no differences in heifers' responses to ACTH challenge were observed between treatments. Repeated regrouping did not affect the growth or reproduction of heifers. These results will be discussed in detail below.

6.1 Experiment 1

6.1.1 Effects of pair vs. individual housing on the welfare of calves

Calves housed in pairs were more active in their home environment: they were more often seen standing, especially moving around. Their higher activity could be due to their pen size, which was twice as large as that of individual pens (4.32 vs. 2.16 m^2). According to Dellmeier et al. (1985) and Jensen (1999), the higher the degree and the longer the duration of confinement of an animal, the greater its activity when released in a larger area. The calves housed individually spent more time running and moved a longer distance (as assessed from the frequency of line crossing) when released in an unfamiliar arena, which was far larger than the home pens (16 m^2). Hence, despite the individual pens being larger than the minimum standards in Europe (Council Directive 97/2/EC), the individually housed calves probably lack sufficient room for movement in the home pen.

Calves housed individually made more contacts (touching, sniffing and licking) through the wooden partitions with their neighbours than the pair-housed calves. However, a higher number of contacts with neighbours did not compensate for the absence of contact with a penmate. Same-pair calves had contact with each other 14.3% of the daytime (i.e. 1 h 43 min), whereas the increase in contact with neighbouring calves observed in calves reared in individual pens accounted for only 2% of the daytime (i.e. 14.5 min). Dellmeier et al. (1985) reported that the lack of social contact leads calves to interact more with conspecifics when given the opportunity to do so. Despite calves being more motivated to attain full social contact rather than only head contact (Holm et al., 2002), no damming-up effect was observed in our individually housed calves when they were with an unfamiliar calf in the arena. Their behaviour towards the animal did not differ from that of calves housed in pairs. Also, during this test their heart rate was similar to that of pair-housed calves, suggesting that they were not more stressed by the presence of a calf despite not being used to such full social contacts. In the preference test, individually housed calves approached the calf that stood at one end of the Y-maze arm less often than did pair-housed calves. Therefore, the visual and physical contact with neighbours through slatted partitions was probably sufficient for individually housed calves not to feel the lack of a penmate.

Calves housed individually had higher cortisol responses to ACTH. The ACTH challenge test included blood samplings and injection by venepuncture. These were done in calves' home pens by a veterinarian. Samplings may have caused distress to the animals because they needed to be held in place by one person while another person took samples or performed the injection. In our experiment, this kind of handling may have been particularly stressful for minimally handled animals. However, no effects of contact conditions were noticed in calves' cortisol responses to ACTH challenge. For pair-housed calves, sampling stress might have been milder than for individually housed because the presence of a penmate may have had a calming effect (Boissy and Le Neindre, 1990). However, our result confirms earlier findings by Dantzer et al. (1983) and Friend et al. (1985), who reported that calves whose movements are limited by tethering or confinement have higher cortisol responses to ACTH. In these earlier studies, calves had very limited contact with other animals; they could see each other and could interact physically with neighbouring calves only through the front of their crate and at feeding times. In these cases, the increase in cortisol responses to ACTH may have been partly due to the reduction of social contact and partly to low space allowance. In our work, by contrast, the calves in individual pens could see, sniff, touch and lick the other calves in adjacent pens through open wooden partitions (10-cm slots) and did not seem to miss the presence of other calves (see above). Hence, even when they have contact with neighbours, calves housed individually seem to be more stressed than calves housed in pairs. However, individually housed calves were in smaller pens (albeit with the same space allowance per animal) than pair-housed calves. Therefore, it is likely that in calves a lack of movement induces chronic stress.

6.1.2 Effects of pair vs. individual housing on calves' responses to people and to handling

Pair-housed calves were hypothesised to be less motivated to interact with people, to be more fearful of people and to be more difficult to handle than individually housed calves. When a person approached their home pen and touched the calves during meal time, calves housed in pairs did not show more avoidance of that person. However, when an unfamiliar person entered their pen, pair-housed calves were slower to interact, interacted less frequently and tended to spend less time interacting with the person in the pen. The latter results seem to support the observation that the interactions of group-housed calves with stock-persons were less efficient, resulting in more difficult handling of group-reared calves than individually reared calves during weighing (Veissier et al., 1998a).

When loaded individually into a truck, it took more time and effort to load and unload pair-housed calves than individually housed calves. This confirms earlier findings that group-housed calves, reared in groups of either 5 or 15, are more difficult to load than their individually reared peers (Trunkfield, 1990; Albright et al., 1991). Albright et al. (1991) reported that group-housed calves exhibited more interest and directed their attention to more sniffing and investigation of the alley floor when loaded into a truck, and Trunkfield (1990) described more balking and turning, compared with individually housed ones. In our experiment, a similar behaviour was observed, with pair-housed calves stopping more often during loading, as indicated by the number of pushes given by the two truck drivers. The reaction to this handling procedure might, however, be confounded by the animals being loaded individually into the truck. Because paired calves were now socially isolated, they may have shown an increased reaction to the handling procedure. However, pair-housed calves were not more stressed, according to the physiological measures of heart rate and cortisol levels, which did not differ significantly between pair-housed and individually housed calves.

During transport individually housed calves had a significantly higher heart rate than their pair-housed counterparts. Different reasons can be given for this. For example, being together with other calves, as it was in the truck, might have led to elevated heart rates for calves that were previously housed individually. Individually housed calves have been reported to lack social experience (Broom and Leaver, 1978), and mixing these calves could cause restlessness or even excitement and an increase in activity (Kenny and Tarrant, 1987), resulting in a rise in heart rate. As for each batch, the four calves of the same treatment were transported together; thus the pair-housed calves were always with their penmate. Therefore, the mixing for the pair-housed calves entailed a lower degree of novelty than for the individually housed calves, who were not used to full social contacts with species companions. Moreover, the presence of a penmate can have a calming effect on the animal's response to a stimulus, as shown by Boissy and Le Neindre (1990), who observed that the presence of peer animals reduced heifers' responses to fear-eliciting stimulations.

6.1.3 Effects of human contact or housing on calves' preferences for other calves and humans

In the preference test, where both an unfamiliar calf and an unfamiliar person were present at the same time with the calf to be tested, no effects of previous human contact were found, whereas large effects of previous housing conditions were noticed. Calves reared in pairs orientated their behaviour towards the calf, while calves housed in individual pens spent the same amount of time near the calf and the man, interacting equally often with both. Therefore, no preference between conspecifics and humans seems to exist in calves reared alone, whilst calves reared in pairs have a clear preference for conspecifics. All calves had been trained to drink from teatbuckets with human assistance. Feeding by humans during the first days of life has a large and long-lasting effect on calves, reducing their fear reactions and increasing the number of contacts with humans (Györkös et al., 1999; Jago et al., 1999; Krohn et al., 2001). Hence, all of our calves may have developed a positive attitude towards humans before the start of the experiment. In calves reared in pairs, full social encounters certainly took place between the two penmates. This probably resulted in a better socialisation of these animals than of individually housed ones, leading pair-housed calves to orientate their behaviour towards the calf in the preference test.

6.1.4 Effects of human contact on calves' responses to people and to handling

The effects of previous additional contact with the stockperson (stroking, talking and letting calves suck fingers at feeding time) were observed in the arena when a man was present. Such previous contact with stockpersons reduced the heart rate of calves when they were subsequently in the presence of an unfamiliar human. This reduction in heart rate can not be accounted for by differences in activity since contact received previously had no effect on the time spent moving in the arena. Activity of animals was also used as a covariate in the analyses. During this test, the calves that had received additional contact spent more time near the man and made more contact with him (sniffing, licking or touching) than did calves that had received only minimal contact. Effects of positive human contact on the behaviour of animals towards humans have earlier been described in calves (Boissy and Bouissou, 1988; Boivin et al., 1998; Jago et al., 1999; Lensink et al., 2000a), sheep (Boivin et al., 2000) and pigs (Hemsworth and Coleman, 1998). Therefore, it is likely that calves that had received positive contact were less fearful in the presence of a human, even when unfamiliar, than calves that had not received this contact.

Improved responses to people were also observed in the transport test. During loading into the truck the additional contact calves had a lower heart rate than calves with minimal human contact. Similar results were found in horses, where animals that had been handled intensively had a lower heart rate during handling than previously non-handled ones (Jezierski et al., 1999), and in sheep, where talking to and patting the sheep reduced the heart rate response to human presence (Hargreaves and Hutson, 1990). The lower heart rate during loading could reflect decreased physical effort connected with movement. However, the level of locomotion (frequency of buck-kicking or time spent running) during loading and the time needed to load the animals did not differ between calves that had received additional contact and those that had not. It is thus more likely that the emotional reaction to presence of and contact with people accounts for the difference in heart rate during loading. Rushen et al. (1999) showed that dairy cows that were previously mistreated by a person had a higher heart rate when this person was present during milking. In our study, it also took less effort to load calves that had received additional contact into the truck compared with minimal contact calves. These results confirm earlier findings in cattle that extra handling, such as brushing or getting them accustomed to being led by a rope, reduces difficulties in handling in routine management practices (Boissy and Bouissou, 1988; Boivin et al., 1992a; 1992b), probably through a reduction in animals' fear responses.

Is positive handling equally effective in pair- and individually housed calves?

We hypothesised that human contacts would be less beneficial for pair-housed calves than for individually housed calves. However, scant evidence emerged for an interaction between contact and housing, except for the tendency of pair-housed minimal-contact calves to be more difficult to unload than all other calves. Thus, human contacts appear to have the same effect in individual and pair housing. However, the lack of interaction between housing condition and contact can be argued to be due to the size of our group. In our study, we housed calves in pairs, but if group size were increased, this would presumably reduce the impact of the stockperson's contacts on individual animals. Furthermore, the calves housed individually in our experiment were not totally isolated tactilly or visually from conspecifics. Total isolation of calves has been reported to induce better coping of animals to human handling through processes similar to "imprinting" (Purcell and Arave, 1991), thereby improving the human-animal relationship. Our individual-housing treatment might not have been sufficiently different from the pair-housing treatment to induce a significant effect in calves' behavioural response to people.

Contacts with humans and contacts with conspecifics seem to have an independent impact on calves since very few interactions between contact and housing conditions were observed. There was one interaction effect observed, on the lying time of calves in their home pen; additional contacts with humans decreased lying time in pair-housed calves while it increased it in individually housed calves, thus enlarging the difference between the two housing conditions. Additional contacts with humans can not therefore be considered an effective means of reducing the stressful effects of single housing because in our experiment it was most probably the lack of movement that caused distress to the individually housed calves. In another experiment (Holm et al., 2002), calves were shown to prefer fuller social contact than merely head contact. Thus, the stressful effect of single housing can either be due to lack of proper space to move or lack of adequate social contact with conspecifics; in our case, it was lack of moving space.

6.1.5 Effects of human contact or housing on calves' production

Although effects of housing and contact were found in behaviour and physiology, no effects were detected on the growth, feed intake or health of calves. Lensink et al. (2000b) found higher growth rates in calves on commercial veal units where farmers exhibited positive behaviour towards their crate-reared animals. However, as in our study, differential responses in behaviour and adrenal activity varied between stalled and penned calves (Delmeier et al., 1985; Friend et al., 1985), but no differences were seen in calves' weight gain. Our treatments, thus, were probably not severe enough to affect calves' health or growth.

6.2 Experiment 2

Repeated changes of penmate and pen alter calves' emotional reactivity by increasing their reactions to novelty, suddenness and fear-eliciting situations (Boissy et al., 2001). Calves' stress physiology is also altered by repeated regrouping; adrenals become hyperreactive and hypersensitive to ACTH, while production remains unaltered (Veissier et al., 2001). Thus, repeated regrouping is a moderate stressor for calves. However, animals of different ages, e.g. calves vs. heifers, have different social motivations and needs (Veissier and Le Neindre, 1989; Veissier et al., 1998b). A stable social environment and bonding with the dam are important for calves (Veissier et al., 1990). Rearing calves in individual crates has, however, no long-lasting effects on their social behaviour (Veissier et al., 1994b). The situation might be different at puberty. Bouissou and Andrieu (1977) found that the social behaviour of heifers develops around puberty. Socially experienced heifers fight less and establish their dominance relations more rapidly than socially inexperienced heifers (Bouissou, 1975). The importance of a diverse social environment at puberty for later socialisation of animals has been reported in guinea pigs by Sachser (1993) and Sachser et al. (1998), who state that the time following puberty is crucial for obtaining the social skills needed to adapt to new conspecifics in non-stressful, non-aggressive ways later in life. Social skills of gilts are also improved by regrouping (van Putten and Buré, 1997). Because our heifers had all reached puberty before the start of the regrouping treatments, they might have been in a critical period of social development, whereas the calves in the studies of Boissy et al. (2001) and Veisiser et al. (2001) had not yet reached this stage.

6.2.1 Effects of repeated regouping on heifers' activity and social behaviour

Regrouped heifers were more active than the controls, but only after the last (16^{th}) regrouping; they moved more and had shorter activity bouts, both signs of agitation. A group of familiar cattle has a calming effect on its members (Boissy and Le Neindre, 1990; Takeda et al., 2003). Our results suggest that this calming effect diminishes when heifers are repeatedly regrouped.

Control heifers were less aggressive among themselves after all observed re-groupings and tended to spent more time close to each other than regrouped heifers after the 5^{th} and 12^{th} regroupings. Previous findings have shown that hei-fers housed together for the first six months of life exchange very few agonistic interactions and form stable preferential relationships (Bouissou and Hövels, 1976; Bouissou and Andrieu, 1978). Preferred penmates stay close to each other, especially during feeding and resting (Bouissou and Hövels, 1976). The early period is known to be the most suitable for complete development of preferential relationships (Bouissou and Andrieu, 1978; Reinhardt and Reinhardt, 1982). In our experiment, regrouping treatments were started when heifers were 11 months old. Thus, heifers had time to form stable relationships before the first regrouping. This explains why control heifers were less aggressive and closer to their permanent penmate than regrouped heifers, who knew each other for only a few days.

No differences in interactions were observed in the social confrontation test be-tween regrouped and control heifers. In the feeding competition test, carried out when heifers were 2.5 years of age, only minor differences between treatments were seen. In addition, these results should be interpreted with caution because we were only able to use four controls and four regrouped animals to test domi-nance relations. Our results thus suggest that extensive regrouping does not modify subsequent social behaviour of heifers.

6.2.2 Do heifers habituate to repeated regrouping?

Pair-housed heifers did not habituate to regrouping. Even after 16 regroupings, regrouped heifers were more active (less time spent lying down and more time standing immobile and sniffing the pen) than control heifers, who stayed in sta-ble pairs. This finding is in contrast to that of Veissier et al. (2001), who re-ported that calves habituated to repeated regrouping by decreasing their immedi-ate social reactivity, measured during three hours after regrouping, when re-grouping was repeated more than nine times. A reasonable explanation for this discrepancy could be related to the sexual maturity of heifers used in this ex-periment, which was controlled prior to regrouping. Bouissou (1977) found that for dairy heifers, adult agonistic interactions, such as fights, butts and threats, substantially increase around the onset of first oestrus. Thus, agonistic behaviour of calves in the study of Veissier et al. (2001) was probably undeveloped, while it was fully developed in the heifers used here. When calves are mixed with new partners, they have been reported to have more non-agonistic than agonistic interactions (Veissier et al., 2001); however, the heifers in our study exchanged both types of interactions at about the same frequency (4-11 interactions per hour). Habituation is a typical reaction to the repetition of a neutral stimulus (Mackintosh, 1987). The lack of habituation of our heifers to regrouping may be due to regrouping not being a neutral stimulus for them. Indeed, it induces aggressive behaviours and may therefore be an adverse experience.

The first regroupings appeared to ease the establishment of dominance relationships in heifers, with the least agonistic interactions and the most rapid development of dominance relationships being observed after the 7th regrouping. These findings are consistent with earlier observations in heifers (Bouissou, 1975). However, after the 7th regrouping, the frequency of agonistic interactions increased again, such that after the 16th regrouping there were more agonistic interactions than in previous regroupings. The establishment of dominance occurred over the longest period after the 16th regrouping; an average of 160 min was required before one of the heifers ceased aggressive behaviours. This time is close to the end of the three-hour observation period. Thus, dominance was probably not established by three hours in most cases. In addition, the dominance relationship was established for only two of the eight heifer pairs within two hours of the 16th regrouping, whereas Bouissou (1974) reported that 84% of such relationships are established within an hour for 18-month-old heifers with no previous regrouping experience. Hence, if the experience of regrouping is necessary to accelerate the formation of a dominance relationship, thereby reducing agonistic interactions, it does not need to be extensive. On the contrary, our results suggest that for pairs of heifers around seven regroupings caused the least agonistic interactions and the most rapid development of dominance relations. Because, variation between successive regroupings was not followed precisely, we do not know if after the 1st regrouping or after the 3rd to 6th, 8th to 12th or 14th to 15th regroupings agonistic interactions would have been lower and dominance relations more rapidly developed than after the 7th regrouping. However, based on earlier studies and our results, no further improvements in these parameters are likely to be obtained when regrouping is repeated extensively, in our case more than seven times.

6.2.3 Effects of repeated regouping on heifers' emotional reactivity

Heifers that had been subjected to repeated regrouping reacted less to the opening of the umbrella than control heifers; compared with controls, more regrouped heifers did not react and fewer displayed strong reactions such as stepping far away from the umbrella. The regrouped heifers also tended to start eating more quickly before the umbrella was opened and started eating more quickly during the arena test. Boissy and Bouissou (1988) found that temporary (3 days/month) prolonged (from 0 to 9 months of age) and repeated human handling of heifers reduces animals' reactivity to unfamiliar and fear-eliciting situations. Hence, repeated events, such as repeated regrouping, seem to reduce the fear reactions of heifers to sudden or novel aspects of the environment.

The regrouped heifers tended to display less activity towards the dog (they tended to be slower to look at the dog and tended to look at it less often and more briefly) and more towards the food (they tended to spent more time near the bucket and eating without looking at the dog). In addition, the changes in behaviour over the sessions were more marked in the regrouped heifers than in the controls. A decrease in the frequency of threats to the dog was observed after the first session in the regrouped heifers but only after the second session in the controls. A similar tendency was observed in the arena test in the absence of the dog; the frequency of eating bouts decreased more rapidly in the regrouped heifers than in the controls. More rapid changes over time could explain why the regrouped heifers were less active and more focused on the food than controls in the dog test. These results suggest that habituation processes occur more rapidly in heifers repeatedly regrouped than in heifers kept in stable pairs.

Rearing animals in a stimulus-poor environment seems to impair their ability to use environmental cues. Animals reared in restricted, small environments are less able to use environmental cues than animals reared in more complex or larger environments (studies on rats and hamsters: Brown, 1968; Thinus-Blanc, 1981; 1982). These findings are confirmed by the results of Varty et al. (2000), who found that rats reared in an enriched environment acquire information from their environment more easily than rats reared in isolation. Pigs housed in an enriched environment learn operant tasks better than their peers housed in a poor environment (Sneddon et al., 2000). Enriching an animal's environment can thus improve its learning abilities. Our control heifers showed slower habituation to behavioural tests and slower behavioural changes from one test to another than the regrouped heifers. Therefore, despite control heifers having an open feeding side in the pen, hearing neighbouring heifers and being taken to be weighed 16 times during the treatments, living in a stable but small group (i.e. a pair) probably formed a more stimuli-poor environment compared with the physically and socially changing environment of regrouped heifers, impairing the control heifers' subsequent ability to habituate in behavioural tests.

In our study, the smallest possible group, a pair, was used. Our data can not, therefore, be generalised to larger groups. However, our regrouped heifers may have felt like they were housed in a larger group, although they met only one new penmate at a time. This assumption would be consistent with the conclusions of Takeda et al. (2003) that heifers' emotional responses to novelty and surprise are lower in groups of five than in groups of two.

6.2.4 Effects of repeated regouping on heifers' stress physiology

The heifers that had stayed in stable pairs had higher cortisol responses to an injection of exogenous CRF than heifers that had been repeatedly regrouped, but ACTH responses were similar. This suggests a difference in the response to CRF between control and regrouped heifers at the adrenal level. Fisher et al. (2002) found that cows that were subjected to restricted recumbency for seven days had an increased plasma cortisol/ACTH ratio following CRF challenge compared with cows that were not restricted. More studies report an increase in adrenal response to ACTH challenge in loose-housed female cattle in response to long-term stressors (e.g. Friend et al., 1977; 1979; Fisher et al., 2002) than a decrease (e.g. Roman-Ponce et al., 1981). We can not, however, offer any conclusions on whether our heifers experienced chronic stress. We ran the CRF challenge test six days after the 15th regrouping. Since acute (3 h following regrouping) behavioural responses to regrouping had later induced agonistic interactions and reduced lying time, regrouping may also have increased heifers' HPA-axis activity. Because changes in HPA-axis activity occur at different levels over time, increased activity right after regrouping might have led to down-regulation of receptor number or affinity six days after regrouping. To confirm the physiological stress responses of our heifers, we should have compared acute responses (i.e. the release of cortisol immediately after each regrouping) with long-term responses (i.e. changes in functioning of the HPA axis).

6.2.5 Effects of repeated regouping on heifers' production

We observed no difference between regrouped and control heifers in growth or immediate reproductive success. Our treatments may not have been sufficiently severe to affect the growth and reproduction of heifers. While differences in behavioural and adrenal activity responses were detected in repeatedly regrouped calves (Boissy et al., 2001; Veissier et al., 2001), no differences were seen in weight gain. Hence, behavioural and physiological modifications in cattle seem to be more sensitive indicators of welfare than productivity, a phenomenon that has also been observed in pigs by Meunier-Salaun et al. (1987).

7 Summary and conclusion

Calves housed in pairs seem less stressed than calves housed individually. This difference does not appear to be affected by social factors in individual housing because calves showed no ''damming-up'' response in social interactions with an unfamiliar calf. Our individually housed calves were able to interact with neighbouring calves in the home pens through the pen walls. The difference in stress reactions was probably due to the greater space available to pair-housed calves than individually housed calves (albeit with the same theoretical space allowance per calf). Pair-housed calves, with the larger space, were more active in their home environment than individually housed calves. The opposite was found when calves were released into an arena. Individually housed calves moved more in the arena, suggesting a discharge of the damming-up of movement in their home environment. These calves sought social contact, and visual and physical contacts with neighbours appeared to be sufficient for calves not to feel the lack of penmates. This contact was not, however, adequate for proper socialisation, as individually housed calves were not motivated to approach other calves in the arena or in the preference test. While regular and positive contacts with humans do not compensate for the lack of species companionship in single housing, they should be maintained to reduce calves' fear of humans.

Calves' reactions to other calves, to people and to handling are influenced by their housing conditions. Pair-housed calves seem to be less eager than individually housed calves to interact with people. Providing regular additional contact in the form of stroking the neck and shoulder of the calves can increase calves' motivation to interact with people, reduce the level of withdrawal, and increase the ease of handling regardless of the housing condition (individually vs. in pairs). Furthermore, calves that are easier to move are less likely to be subject to rough handling, which in turn improves welfare. Group-housed animals should therefore receive positive contacts during rearing to overcome fear of people, increase ease of handling and decrease the stockperson's workload and risk of injury.

Although repeated regrouping causes more agonistic interactions among heifers and increases the distance kept between animals (after 5th and 16th regrouping), it lowers heifers' emotional reactivity to novelty, suddenness, and fear eliciting situations in comparison with rearing heifers in stable pairs. While lower reactivity is undesirable for fitness of prey animals in the wild, it may be an advantage for production animals, which are handled by humans. The effects of repeated changes of partners and pens on heifers were confusing. Social skills of heifers are likely developed around puberty. Diversity rather than stability of the social environment thus appears to be more beneficial to heifers in modern dairy husbandry, particularly when the rearing group has previously been small.

Implications for farming

In calves, group housing is superior to housing animals individually. Calves should receive positive contact from stockpersons; this contact is especially important for group-housed calves to enable them to better habituate to humans.

Our studies show that, contrary to calves, heifers housed in pairs benefit from broader social experiences. We thus recommend regrouping heifers a few times to prepare them for integration in a herd of adult cows.

Future studies

The following questions require further studies:

a) What is the role of familiar social partners in individual behaviour of cattle? Other questions arising from this are: at what age and how the preferential relationships between calves develop, what is the importance of preferred partners to individual animal, and how permanent can these relationships be? Social behaviour of cattle reared together since calves should be recorded as well as their reactions to later peers.

b) What is the effect of group size on human-cattle interactions? Other questions arising from this are: how many individual cattle is a qualified stockperson able to properly attend to in group-rearing systems, and how much does the effect of positive human handling differ between group-housed and individually housed cattle? These questions could be answered by studying the interaction between human contact and group size (from 1 to 2 or more individuals).

c) What is the relationship between acute stressors, such as regrouping, and chronic stress in social situations? Behaviour and cortisol responses after each regrouping should be recorded and cortisol responses compared with responses to ACTH/CRF challenges.

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