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Does repeated regrouping alter the social behaviour of heifers?

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Abstract

Regrouping of cattle according to age, live weight or production stage is a common practice on farms. Our study aimed at determining how repeated regrouping modifies the social behaviour of dairy heifers, and more specifically if dominance relationships are formed more rapidly when animals become more familiar with regrouping. Thirty-two Holstein heifers were housed in pairs. From the age of 11 months, half of the heifers were placed in a new pen with a new penmate once or twice weekly for a total of 16 times over 11 weeks (regrouped heifers), while the other half remained in the same pen with the same penmate (controls). The heifers were monitored (a) for 3 h immediately after the 2nd, 7th, 13th and 16th regrouping, (b) for 24 h before the 1st regrouping, and after the 5th, 12th and 16th regrouping and (c) during a social confrontation test (with one animal for each treatment placed together in an arena) just before the 16th regrouping. Regrouping consistently induced agonistic interactions. Heifers exchanged the fewest agonistic interactions on the 7th regrouping and the greatest on the 16th. Dominance relationships were established most rapidly on the 7th regrouping (84 ± 20 min), but developed over the longest period on the 16th regrouping (158 ± 56 min). Observations for 24 h after the 16th regrouping showed that regrouped heifers moved and changed their activity more often than the controls. No treatment differences were observed in the social confrontation test. In conclusion, heifers do not habituate to regrouping and repeated regroupings have little effect on their subsequent social behaviour. An optimum of around

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seven regroupings appear to produce the fewest agonistic interactions and allow dominance relationships to be established more rapidly.

Keywords: Heifer; Social behaviour; Regrouping; Dominance

1. Introduction

In feral cattle, cows, heifers and calves co-habit within a large herd and new members are rarely accepted into the established group (for a review Bouissou et al., 2001). However, dairy calves are separated from the dam soon after birth (within a day to several weeks after birth) and usually reared in groups with other calves born during the same period. Thereafter, heifers of the same year are generally reared together until first calving, before or after which they are integrated into main dairy herd where they can be exposed to further regroupings according to milk yield (Arave and Albright, 1981; Konggaard et al., 1982). Some heifers may also be sold. In contrast to feral cattle, young dairy heifers often undergo many changes in their social environment.

Calves (4.5 months of age) and heifers (18 months of age) with prior experience of regrouping form more stable relationships, fight less and establish dominance relationships more rapidly than animals having no such experience (Bouissou, 1975; Veissier et al., 1994). In addition, regrouping experiences seem to improve subsequent social behaviour of cattle: 4.5-month-old calves that have been in groups early in life are found at the top of the hierarchy when regrouped with calves of similar age that have been reared in isolation (Veissier et al., 1994). However, calves of the same age having experienced one regrouping (i.e. housed in individual crates for 3.5 months then regrouped) dominate calves that have been maintained as a group (Veissier et al., 1994). Hence, there may be an optimum level of social experience, which when exceeded could have detrimental effects on the social behaviour of cattle.

The social behaviour of cattle can also vary with age and maturity. In the first months of life, calves exchange few aggressive interactions, which tend to be more playful and bi-directional, and calves do not form a clear social hierarchy (Bouissou et al., 2001; Canali et al., 1986; Reinhardt and Reinhardt, 1982). In addition, calves are not particularly aggressive to each other after regrouping compared with semi-mature or adult cattle. For instance Veissier et al. (2001) reported less than 2 aggressive interactions between calves during the first 3 h following regrouping whereas Bouissou (1974) observed 10 aggressive interactions of heifers within the first hour of regrouping. Calves also habituate to regrouping, whereby they are less and less agitated when regrouping is repeated (Veissier et al., 2001). After puberty, dominance related behaviours and adult type agonistic interactions, such as butting and threatening behaviour become more prevalent (Bouissou, 1977). Therefore, regrouping would be expected to affect semi-mature heifers' more than young calves.

In addition to the dominance hierarchy, the social organisation of cattle is characterised by affinity bonds that are responsible for the cohesion of a group (Arnold, 1985). In calves, these bonds are strongly influenced by the time animals have been together (Ewbank, 1967).

In this paper, we examined the extent to which repeated regrouping alters the social behaviour of heifers following further regroupings (aggressive behaviour and affinities). More specifically, this work tested the regrouping of heifers based on two alternative working hypotheses namely: (1) heifers may habituate to regrouping, in which case they would be less and less agitated as regrouping is repeated or (2) as heifers acquire more experience of regrouping, dominance relationships may be formed more and more rapidly, resulting in less and less agonistic interactions.

2. Materials and methods

The experiment was conducted at the experimental farm of INRA at Marcenat (Cantal, France). Based on French and Finnish regulations, the scientists involved in the experiment (I. Veissier and A. Boissy in France, and S. Raussi in Finland) are licensed to perform such experiments on animals, while the personnel responsible for rearing the animals and collecting samples have completed approved training courses.

2.1. Animals, housing and experimental treatments

Thirty-two Holstein Friesian female calves born in October 2000 were used. Ten calves were from the INRA Marcenat experimental farm and 22 from the INRA experimental farm of Les Monts Dore (Puy de Dôme, France). Soon after birth, calves were transported to the calf experimental barn. Calves were housed in pairs in 1.8 m × 2 m pens separated by solid wooden partitions and bedded with straw. The temperature in the barn was maintained between +12 and +20 °C and constant lighting was provided between 08:00 and 18:00 h. The calves were fed milk replacer (Univor energie, Centraliment France) and hay. They were weaned from milk at 12 weeks of age. The pairs of calves were allocated to two treatments (regrouped versus control, see below) so that their date of birth, live weight at parturition and farm of origin were similar between treatments. At 6 months of age, paired animals (termed heifers thereafter) were moved to a second barn into pens (4 m × 5 m) fitted with 2 m high solid wooden partitions and bedded with straw. A hayrack and a feed trough for concentrates were placed on one side of the pen. The animals were fed hay (10 kg/day/animal) and concentrates (Gala elevage, Centraliment France, 2.5 kg/day/animal) between 08:00 and 09:00 h each day. Stockpersons checked the health of the animals twice daily and appropriate medical treatments were administered where necessary. On reaching 10 months of age, heifers were blood sampled and progesterone assays were performed on two occasions with a 10-day interval in between to ensure that each heifer had reached puberty. Because some heifers were not cycling a treatment had to be administered to induce oestrus. Heifers 10.5 months of age, received an IM injection of 3 mg Norgestomet (17-alpha-acetoxy-11-beta-methyl-19-norpreg-4-en-3.20-dione) and 3.8 mg oestradiol and were given a subcutaneous 3 mg Norgestomet implant in the ear that was maintained for 10 days. All heifers, irrespective of cyclic activity were implanted to remove bias in the experiment.

Eight pairs of heifers were kept in the same pens from the beginning to the end of the treatment period (controls). For the remaining eight pairs, heifers were housed in different

pens and different penmates repeatedly from the age of 11 months (regrouped heifers). To achieve this, each heifer was weighted separately, with control animals being returned to the original pen, while regrouped heifers were housed in different pen with an unfamiliar heifer from the same treatment group. Regrouped heifers remained in the new pen with the new penmate until the next regrouping. The weighing–regrouping procedure was performed between 14:00 and 16:00 h, being repeated twice weekly for 5 weeks, then once a week for the next 6 weeks, giving a total of 16 regroupings. From regroupings, 13 to 16, 19 pairs of heifers were comprised of animals that had prior experience of each other. However, at least 10 regroupings separated the two occasions on which the same animals met. To avoid resident–intruder effects, regrouped animals were never housed in a pen for which they had been in during the previous regrouping.

2.2. Measurements

Four cameras were fixed to the ceiling of the barn with each camera recording four pens (i.e. eight heifers). A fifth camera was fixed above the arena and used for the social confrontation tests (see below). Each camera was connected to a videotape recorder. The behaviour of the heifers was further encoded on a computer using the Observer Video Pro programme (Noldus, The Netherlands).

2.2.1. Behaviour following regrouping

The behaviour of heifers immediately after regrouping was monitored to establish reaction to regrouping. The behaviour was recorded for 3 h directly after the 2nd, 7th, 13th and the 16th regrouping. Recordings were started after the two animals were introduced into a pen. The following activity states were distinguished: lying down, standing immobile, moving, eating (defined as the head being in the feed trough or in the hay track), licking salt and drinking. These states were mutually exclusive. The frequency and duration of individual states were calculated. Interactions between heifers were recorded as events. The interactions were classified as: fight (animal heads touching and pushing against each other), an efficient butt (violent contact of the head or horn from another animal with the recipient turning away), a non-efficient butt (same as an efficient butt but the receiver does not move away), efficient threat (the same movement as a butt but with no contact and the recipient turning away), non-efficient threat (same as an efficient threat but the receiver does not turn away), flight (animal turning the head or moving away when another approaches without any threat or butt), sniffing the penmate apart from the anogenital area, sniffing the anogenital area of the penmate, head against head (animals standing head to head without pushing), head play (animals rubbing heads against each other), head against body (one animal having the head against the penmate's body but not pushing), small butts (same as butt but not violent and often repeated), pushing (one animal having the head against the other animal's body and pushing), licking the penmate in areas other than the anogenital area, licking the anogenital area of the penmate, head on the bottom or back of the penmate, mounting, flehmen (upper lip reversed) and sniffing the pen. Events were grouped to agonistic and non-agonistic behaviours according to Bouissou et al. (2001) to calculate the latency and frequency of efficient agonistic interactions (efficient threat, efficient butt, fight and flight), non-efficient agonistic interactions (non-

efficient threat and non-efficient butt), total agonistic interactions (either efficient or non-efficient), non-agonistic interactions (head against head, head play, head against body, small butts, pushing and licking or sniffing apart from the anogenital area) and sexual interactions (sniffing or licking the anogenital area, head on the bottom or back of another animal, mounting and flehmen).

For regrouped heifers, the time when a dominance relationship was established was determined as follows: when two heifers were regrouped, aggressive behaviours (butts, threats and fights) could be displayed by one or both animals; but after a while this was expressed by only one heifer (dominant) and the other animal displayed flight behaviours (subordinate). The time (in minutes) after which one of the two heifers ceased to display aggressive behaviours and tended to express submissive behaviours was the time at which a dominance relationship was considered to be established.

2.2.2. Behaviour over 24 h

Heifers behaviour over 24 h was followed to establish if repeated regrouping had an effect on spontaneous social behaviour after the dominance relationship was formed. Animals were observed before the 1st regrouping and 2 days after the 5th, 12th and 16th regroupings. Five-second scans were taken every 5 min for 24 h. Two classes of behavioural states were observed: activity and proximity. The activity states were the same as for observations following regroupings (see Section 2.2.1). Proximity included three states: animals in contact with each other (bodies touching), animals not in physical contact but at a distance less than or equal to 1 m and animals at a distance of more than 1 m. For each class, states were mutually exclusive. The percentage of time spent on a given activity or proximity state, the number of changes of activity and the mean duration of activity and proximity bouts were calculated for individual animals on each observation day.

2.2.3. Social confrontation test

A social confrontation test was performed to find out if further social responses towards a stranger differed between regrouped and control heifers. This test was carried out for all heifers in an arena 1 day before the 16th regrouping. The arena was situated in the barn where the heifers were housed and measured 5.4 m $\times$ 7.1 m, with a ground floor surrounded by 2.2 m high wooden walls. Excreta were removed from the arena floor after every test. 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 for the other half. After 4 min, a bucket of concentrates 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 (for description see Section 2.2.1).

2.3. Statistics

Statistical analysis was performed using SAS (version 6.12, SAS Institute Inc., USA; SAS, 1989). When a Gaussian distribution and homogenous variance of residuals was confirmed, the General Linear Model (GLM) was used to assess treatment effects. To account for group effects, animal pairs were used as a random factor for all data collected in

the home pens (except proximity and time to establish dominance for which there were only single observations per pens) and treatment effects were evaluated against pair effects. Since animal pairing was changed by regrouping, data could not be analysed by ANOVA for repeated measures. However, to assess whether heifers learned to form a dominance relationship as the number of regrouping increased, within animal comparisons of the time necessary to establish a dominance relationship was performed by considering individual regrouped animals as the unit of observation and running an ANOVA for repeated measures. Means and standard errors are presented. Effects were considered significant at $P < 0.05$ and $P < 0.10$ were considered as a trend.

3. Results

3.1. Behaviour following regrouping

After each regrouping, regrouped heifers sniffed their pen more frequently than the controls (Fig. 1a; $F_{1,14} \geq 5.31$, $P < 0.05$), spent more time standing immobile (Fig. 1b; $F_{1,14} \geq 9.45$ after the 2nd and 7th regroupings and $F_{1,14} = 3.36$ after the 16th regrouping, $P < 0.01$ and $P = 0.09$), and less time lying down (Fig. 1c; $F_{1,14} \geq 5.88$ after the 2nd and 7th regroupings and $F_{1,14} = 3.40$ after the 16th regrouping, $P < 0.05$ and $P = 0.09$).

Regrouped heifers exhibited agonistic interactions more rapidly and at a higher frequency than the controls who expressed few agonistic interactions (Fig. 2a and b; latency of first agonistic interaction: $F_{1,14} \geq 21.0$, $P < 0.001$; Frequency of agonistic interactions: $F_{1,14} \geq 10.4$, $P < 0.01$). Agonistic interactions were generally efficient in regrouped heifers and inefficient for the controls: after the 2nd, 7th, 13th and 16th regroupings, 91 ± 2.58, 92 ± 3.24, 86 ± 6.18 and $94 \pm 1.49\%$ of agonistic interactions were efficient in regrouped heifers versus 10 ± 7.28, 6 ± 6.25, 11 ± 6.93 and $33 \pm 11.4\%$ in controls ($F_{1,14} \geq 20.7$, $P < 0.001$). After the 7th regrouping, regrouped heifers exchanged more non-agonistic interactions than the controls (Fig. 2c; $F_{1,14} = 12.2$, $P < 0.01$) and exchanged more sexual interactions after the 7th regrouping (20.0 ± 5.32 versus 1.13 ± 0.43; $F_{1,14} = 9.78$, $P < 0.01$), which tended to continue for the 16th regrouping (7.50 ± 1.65 versus 17.6 ± 3.38, $F_{1,14} = 4.10$, $P = 0.06$).

Dominance relationships were established the most quickly after the 7th regrouping (84 ± 20 min versus 134 ± 17 min; 121 ± 22 min and 158 ± 56 min after the 2nd, 13th and the 16th regroupings). The time taken to establish a dominance relationship was significantly lower at the 7th regrouping than 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 ($F_{1,15} = 3.73$, $P = 0.07$).

3.2. Behaviour over 24 h

Results for heifers activity and proximity over 24 h before the 1st regrouping and after the 5th, 12th and 16th regroupings are presented in Table 1. Before the 1st regrouping, there were no differences in activity or proximity between animals. After the 5th regrouping,

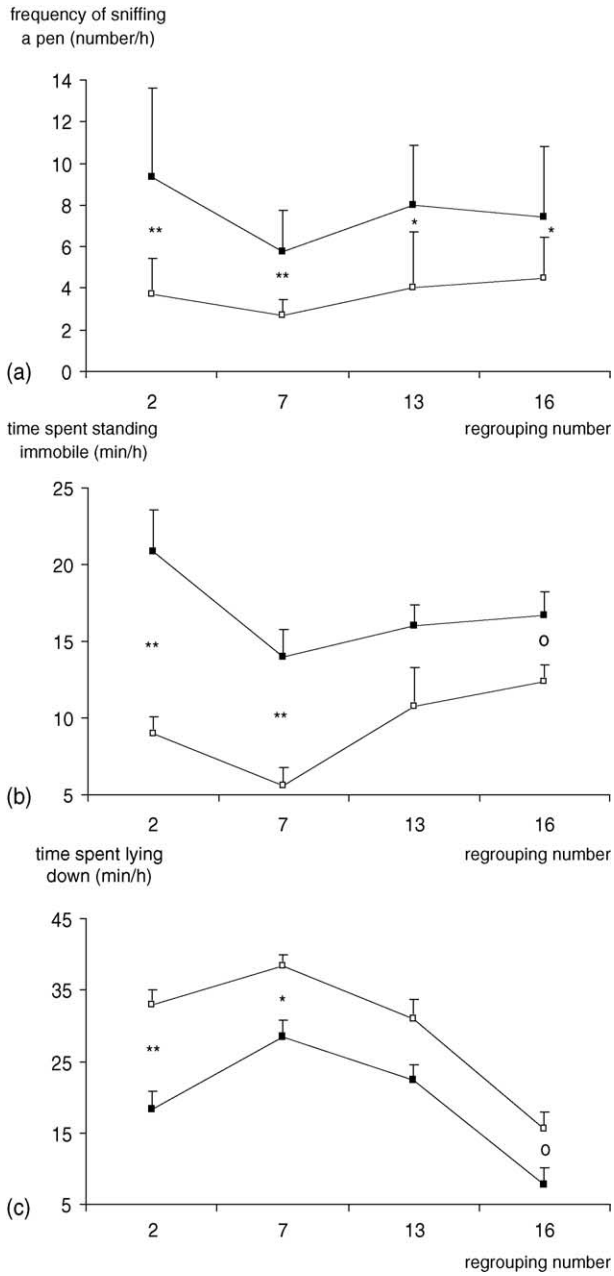

Fig. 1. General activity of heifers during the 3 h following a regrouping 2, 7, 13 and 16. Regrouped heifers ($n = 16$, ■) are compared with controls ($n = 16$, □). (a) Frequency of sniffing a pen; (b) time spent standing immobile; (c) time spent lying down. ANOVAs were run at each time point to compare treatments; $^{\circ}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. F-values are given in the text.

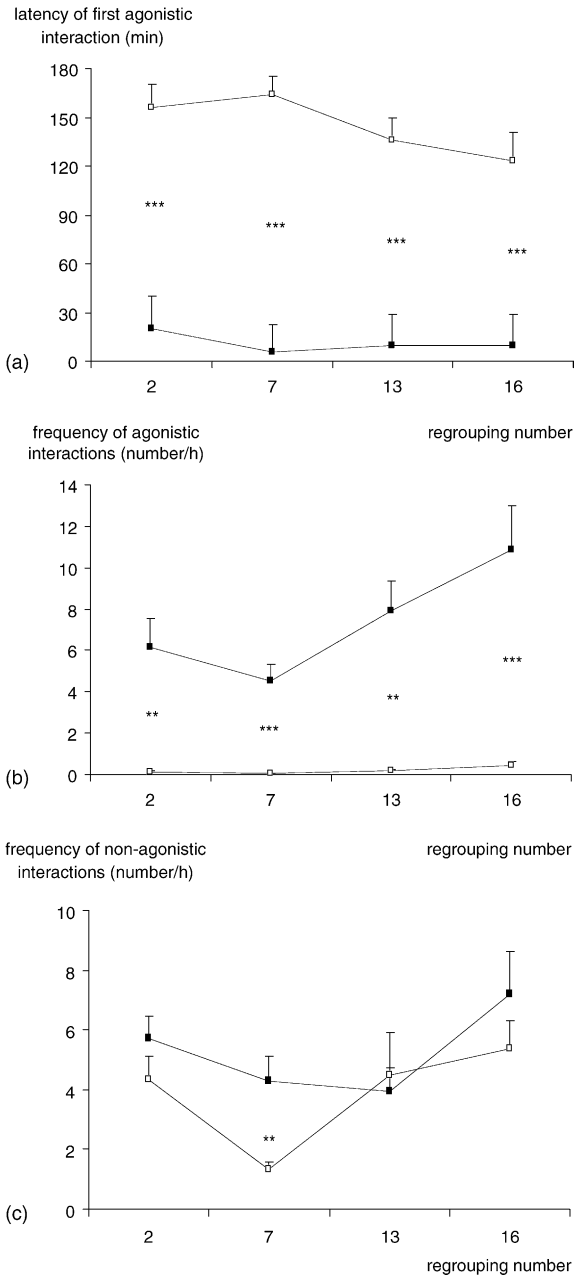

Fig. 2. Agonistic and non-agonistic interactions of heifers during the 3 h following a regrouping 2, 7, 13 and 16. Regrouped heifers ($n = 16$, ■) are compared with controls ($n = 16$, □). (a) Latency of first agonistic interaction; (b) frequency of agonistic interactions; (c) frequency of non-agonistic 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.

Table 1
Behaviour over 24 h for control (not regrouped) and regrouped heifers before the 1st regrouping and 2 days after the 5th, 12th and 16th regroupings

	Mean		S.E.	$F_{1,14}$	P
	Control $n = 16$	Regrouped $n = 16$			
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

regrouped heifers tended to be less frequently in contact with each other and the duration of contact bouts was shorter than for the controls. After the 12th regrouping, regrouped heifers tended to be over 1 m away from each other more often than the controls. After the 16th regrouping, regrouped heifers moved and changed activity more often with shorter activity bouts than the controls.

3.3. Social confrontation test

During the social confrontation test, heifers performed on average 11.7 (±1.12) agonistic interactions, 2.75 (±0.44) non-agonistic interactions, 3.25 (±0.96) sexual interactions and ate for 1.54 min (±0.17), with no significant differences between treatments.

4. Discussion

In this study, heifers did not habituate to regrouping. After regroupings 2, 7 and 16 regrouped heifers were more active (less time spent lying down and more standing immobile and sniffing the pen) than control heifers. Hence, heifers still responded to regrouping even on the 16th occasion. These findings are in contrast with those of Veissier et al. (2001) who reported that calves appeared to habituate to repeated regrouping. A reasonable explanation for this discrepancy could be related to the sexual maturity of heifers used in this experiment, which was controlled prior to regrouping. Bouissou (1977) found that for dairy heifers, adult types of agonistic interactions, such as butts and threats, increase substantially 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 heifers used in the current study. When calves are mixed with new partners, they exchange more non-agonistic than agonistic interactions (Veissier et al., 2001) while the heifers in this study exchanged both interactions at about the same frequency (4–11 interactions per hour). The habituation process includes a decline in response to a neutral stimulus that is repeatedly presented (Mackintosh, 1987). The lack of habituation of heifers to regrouping may be due to regrouping not being a neutral stimulus, since it induces aggressive behaviours, and may therefore be an adverse experience.

The first regroupings appeared to ease the establishment of dominance relationships, with the least agonistic interactions and 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 started to increase, such that after the 16th regrouping there were more agonistic interactions compared with previous regroupings. The lower level of aggression on the 7th regrouping cannot be attributed to familiarity of animals, because on the 7th regrouping no regrouped animals had previous experience of each other, whereas on the 13th regrouping two animals had met before, while all regrouped heifers on the 16th regrouping had met before. Besides, the establishment of dominance occurred over the longest period after the 16th regrouping. After the 16th regrouping, an average of 160 min was required before one of the pair of heifers ceased aggressive behaviours. This time period is close to the end of the 3 h observation period. Thus, dominance was probably not established in most cases. Further, the dominance relationship was established for only two of the eight heifer pairs, within 2 h following the 16th regrouping, whereas Bouissou (1974) reported that 84% of such relationships are established within an hour for 18-month-old heifers. Hence, if the experience of regrouping is necessary to accelerate the formation of a dominance relationship, and thereby reduce agonistic interactions, it does

not need to be extensive. Our results suggest that for groups of two heifers, an optimum experience may be around seven regroupings. However, variation between successive regroupings was not followed precisely, and therefore it can only be suggested that a true optimum lies between 3 and 12 regroupings, and that no further improvements are likely to be obtained when regrouping is extensively repeated.

Control heifers were less aggressive among themselves and spent more time close to each other compared with regrouped heifers. This was seen in the 24 h following the 5th and 12th regroupings. Previous findings have shown that heifers housed together for their first 6 months of life exchange very few agonistic interactions and form stable preferential relationships (Bouissou and Andrieu, 1978; Bouissou and Hövels, 1976). Preferred penmates stay close to each other especially during feeding and resting (Bouissou and Hövels, 1976). It is well known that the early period is the most suitable for the 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 of age. Thus, heifers had time to form stable relationships before the first regrouping. This explains why control heifers were closer to their permanent penmate than regrouped heifers, who knew each other for only a few days.

Regrouped heifers were more active than the controls, but only after the last 16th regrouping: they moved more, had shorter activity bouts and changed their activity more frequently. 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 animals are repeatedly regrouped.

No differences were observed in social confrontation tests between regrouped and control heifers. At the end of the experiment, eight heifers (four controls and four regrouped) were maintained as a group and reared together until calving. On reaching 2.5 years of age, pairs of one regrouped and one control heifer were subjected to a feeding competition test using the methodology developed by Bouissou (1977) (results not reported here). No differences in the interactions initiated by heifers from the two treatments were observed. Thus, extensive regrouping, or rearing for one year with the same penmate, does not appear to modify the subsequent social behaviour of heifers.

In conclusion, in pairs of heifers, a dominance relationship was most rapidly established after the 7th regrouping with the fewest agonistic interactions and a reduction in subsequent activity level, while the opposite was true after the 16th regrouping. Therefore, there seems to be an optimum amount of social experience after which an increase in regrouping experience does not appear to assist the establishment of dominance relationships, and may even alter it. A social hierarchy helps to reduce conflicts and fighting (Bouissou and Signoret, 1970), and therefore it is recommended that heifers are regrouped on several occasions to prepare them for integration into the main adult dairy herd.

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