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Behaviour, wounds, weight loss and adrenal weight of rabbit does as affected by semi-group housing

Stephanie Buijs[a,*], Luc Maertens[a], Katleen Hermans[b], Jürgen Vangeyte[c], Frank André Maurice Tuyttens[a]

[a] Animal Sciences Unit, Institute for Agricultural and Fisheries Research (ILVO), Scheldeweg 68, Melle, Belgium
[b] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke, Belgium
[c] Technology and Food Science Unit, Institute for Agricultural and Fisheries Research (ILVO), Van Gansberghelaan 115, Merelbeke, Belgium

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ABSTRACT

Group housing is often assumed to improve the welfare of gregarious species. Whether this is actually the case depends on the advantages (e.g. more opportunity for social and locomotor behaviour) and disadvantages (e.g. increased fighting and wounding) induced by the specific housing type. We evaluated the effects of a semi-group system (grouping four does for half of each reproductive cycle) on welfare by comparing it to single-doe cages. Compared to this control, our semi-group system provided more total space when does were grouped and more space per doe (a confounding deemed necessary to avoid overt aggression). Thus, the results should be interpreted as a systems comparison. In each of the four experimental cycles semi-group does were housed separately for 21 days around parturition and housed in newly assembled groups for the next 21 days. Behaviour was observed in semi-group and single-doe systems immediately after the second time semi-group does were mixed, and during five timeslots divided over the second experimental cycle. Skin lesion and weight loss were determined in each cycle. Adrenal weight was measured post-mortem. Semi-group systems with different floor types were included but floor type effects were scarce and semi-group systems were therefore treated as one category. In the timeslot subsequent to mixing semi-group does spent a greater percentage of their time on locomotion (4.3 vs. 0.7%, $P<0.01$) and social sniffing/grooming (1.4 vs. 0%, $P<0.01$) than does in single-doe cages. Such differences also occurred in later timeslots, but were much smaller (e.g. midnight locomotion D12: 0.8 vs. 0.2%, $P<0.05$, midnight social sniffing/grooming D12: 0.4 vs. 0%, $P<0.01$). Attacking/chasing followed a similar pattern (following mixing: semi-group 5.3% vs. single 0%, $P<0.01$; midnight D12: 0.01 vs. 0%, $P<0.10$). A high percentage of semi-group does were slightly (58%) or severely (20%) wounded. Semi-group does spent a smaller percentage of the timeslot following mixing in bodily contact with adults than does from single-doe housing (who could only make contact through the wire walls, 1.6 vs. 11.8%, $P<0.01$). Even 12 days after mixing the percentage of time semi-groups spent in bodily contact did not exceed that in singles ($P>0.10$). In experimental cycle one only, semi-group does lost more weight during late lactation than singles (192 vs. 10 g, $P<0.01$). Adrenal weights did not differ between systems ($P>0.10$). Further research will be needed to design semi-group systems with a more favourable balance between advantages and disadvantages.

1. Introduction

Group housing of gregarious animals increases opportunities for social behaviour and behaviours facilitated by more total space. However, group housing often means that animals are forced into groups without being able to switch between or retreat from these. Furthermore, animals are often regrouped to facilitate husbandry efficiency. Such forced membership of unstable groups may lead to fighting and social stress (Noller et al., 2013; Paredes et al., 2006). Although providing more (total) space than individual housing, group pens still restrict animals to a much smaller area than used by their wild conspecifics (e.g. home ranges of wild female rabbits are reported to vary between 600 and 8000 m^2, Myers and Poole, 1961, whereas group systems usually offer between 1.5 and 9 m^2, Rommers et al., 2006; Stauffacher, 1992). Whether social and spatial restraints become problematic in group housing depends,

* Corresponding author.
E-mail address: stephanie.buijs@ilvo.vlaanderen.be (S. Buijs).

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amongst others, on the reasons for social attraction in the wild, the animals' ability to thrive in any kind of group and the underlying reason for space use in the wild. For instance, animals with a strong motivation to explore or patrol a large home range are likely to suffer when kept in spatial restriction (Clubb and Mason, 2007). However, if a species' space use is mainly driven by acquiring enough nutrients, as for instance in wild rabbits (Lombardi et al., 2007), it is unlikely to reflect space needs in captivity where food is supplied ad libitum. Similarly, group formation in the wild is not always the result of high sociality (Macdonald, 1983) whereas specifically highly social animals are likely to suffer from individual housing. As such, whether or not group housing actually improves welfare depends on the species as well as the system.

Reproduction does used to breed meat rabbits are commonly housed "individually". Such housing is not truly individual, as kits are present most of the time. Therefore it is called "single-doe housing" in this article. Single-doe cages (usually between 2400 and 4000 cm^2) likely to restrict does' locomotion severely, as does can traverse the full cage length in a single hop (EFSA, 2005). They also limit inter-doe physical contact to tactile contact through the wire walls. This contrasts sharply with natural conditions, where does live communally (Mykytowycz and Rowley, 1958). Non-breeding laboratory rabbits prefer large group pens over small individual ones (Held et al., 1995) and can be grouped without causing overt aggression (Fuentes and Newgren, 2008; Held et al., 1995; Turner et al., 1997) or stress (Whary et al., 1993). Designing group systems for breeding rabbits is more difficult, as these are constantly either pregnant or lactating (and often both). Wild rabbits usually kindle and suckle their young away from their social group (Mykytowycz and Rowley, 1958) and breeding rabbits may also want to leave their groups at such times. Furthermore, does are more aggressive when close to their nests (Rödel et al., 2008). Due to space limitations in most group systems does are generally close to their nest, increasing the likelihood of social stress and wounding. Andrist et al. (2013) report that on commercial farms, 34% of group housed does had wounds.

In semi-group housing does are separated a few days before kindling to be grouped again 2–3 weeks later. This means that does cannot fight at the time of the cycle they are most likely to do so, and that they cannot destroy each other's young, vulnerable litters (a behaviour observed both in wild (Künkele, 1992; Rödel et al., 2008) and commercial does (Szendro and Mcnitt, 2012)). When regrouped, does have access to more space and adult social partners. Data on how such systems affect welfare are scarce. Repeatedly separation and regrouping may cause social stress, especially when group composition varies (which is likely in practice). Also, separating does around kindling may actually increase aggression at regrouping (Andrist et al., 2013). Rommers et al. (2014) found that 52% of semi-group housed does were wounded.

We studied behaviour, wounding, weight loss and adrenal weight of does in single-doe housing and in semi-group housing. Semi-group housing was hypothesized to increase affiliative and locomotor behaviour, but also to increase agonistic behaviour and wounding. For weight loss and adrenal weight two-sided hypotheses were formulated. These might decrease in semi-group housing due to a more appropriate social situation, or to increase due to forced membership of an unstable group. Weight loss could also increase due to additional exercise or reluctance to feed.

2. Methods

All procedures were approved by the ILVO ethical committee for the use of animals in research.

The study only evaluated doe welfare, without assessing the impact on the welfare of the kits. Effects on kit temperament are described in Buijs and Tuyttens (2015).

Table 1
Overview of husbandry procedures and data collection during the 42-day long reproduction cycles.

Days post-kindling	Procedure
−3	Does moved to new cage or new separate unit of semi-group pen
0	Kindling
11	Insemination[a]
18	Wound scoring and weighing (baseline) Grouping of does in semi-group housing treatments Daytime video recording for behavioural analysis[b]
19	Nighttime video recording for behavioural analysis[b]
22	Nighttime and daytime video recording for behavioural analysis[b] Wound scoring
30	Daytime video recording for behavioural analysis[b]
31	Nighttime video recording for behavioural analysis[b]
32	Weighing and weaning (does moved to other room in existing groups)
39 = −3	

[a] Does were not inseminated in the last cycle.
[b] Cycle 2 only.

2.1. Animals and husbandry procedures

Seventy-two 29-week-old Hycole does (Hycole, Marcoing, France) were allotted randomly to one of three housing treatments (see below) 3 days before their second kindling. The does remained in their treatment for four consecutive reproduction cycles, although they were moved to another cage or pen twice per 42-day-long cycle (Table 1): at 32-days post-kindling they were moved to wean their young (which remained in their native cage or pen at this time), and at 39 days post-kindling they were moved to form new groups of unfamiliar does for the next experimental cycle. These new groups of unfamiliar does were created because not all does became pregnant upon insemination, and these does were replaced with animals from a spare compartment (within treatment). This follows the procedure in commercial rabbit husbandry, where non-pregnant does are moved to different production groups. To prevent heterogeneity in activity between our groups resulting from the replacement of non-pregnant does in some of the groups, all does were moved to a different cage or pen to create new groups of unfamiliar does in each pen each cycle. Animals in single-doe housing were also moved to a new cage at weaning and at 39 days post-kindling (when they were placed next to unfamiliar does) to avoid the incorrect attribution of the effect of moving itself to the housing system. Conditions in the spare compartment were the same as in the experimental room (semi-group housing or single-doe housing on wire or plastic according to each doe's experimental treatment, same space allowance and light and temperature regimen). Does had ad libitum access to a commercial pelleted rabbit feed (17.0% crude protein, 16.2% crude fibre and 10.3 MJ digestible energy). However, non-pregnant, non-lactating animals in the spare compartment were limited to 140 g per day to prevent obesity. Water and a simple cage enrichment (a wooden gnawing block fixed to the side wall of the cage or pen) were available continuously to all does. Underpressure ventilation and a central heating system were used to achieve a stable climate (mean temperature 16.9 ± 2.1 °C SD, mean relative humidity 56 ± 9% SD).

2.2. Housing treatments

Three housing treatments were included in the experiment: single-doe cages with a wire floor, semi-group pens with a wire floor and semi-group pens with a plastic slatted floor. Twenty-four does were housed in each housing system. Bodyweight at the start and end of the experiment did not differ significantly between the treatments (start: $F_{1,70} = 0.03$, $P = 0.85$, mean: 4.7 kg ± 0.4 SD, end:

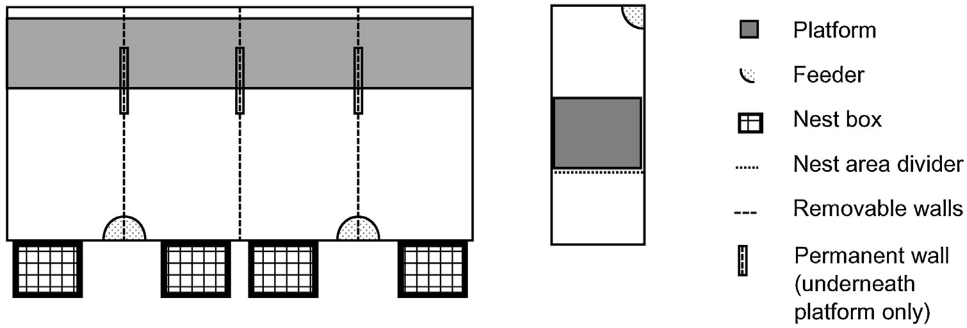

Fig. 1. Schematic top-view of the semi-group system (left) and the single-doe cage (right).

$F_{1,68}$ = 0.51, P = 0.48, mean 4.8 kg ± 0.3 SD). The housing types were homogeneously distributed over each of two experimental rooms which were used alternately (i.e. all does were moved to the other room at weaning where they remained for the duration of their next cycle).

Each single-doe cage (Meneghin s.r.l., Povegliano, Italy) had a floor area of 38 cm × 104 cm (Fig. 1). However, from 3 days prior to kindling until weaning at 32 days post kindling an area of 29 cm × 38 cm in the front of the cage was separated from the rest of the cage and used as a nest box, as is commercial practice. Although does were able to enter their nest box freely, most spent little time in the nest box once they had kindled, and in any case the insertion of the separator segmented the available cage area. In addition to the floor level, an elevated platform of 38 cm × 30 cm was available to the does. The height of the cage varied: 28 cm in the nest box, 28 cm underneath the platform, 35 cm above the platform and 63 cm in the back of the cage. The floor and platform were made of 2.5 mm wide metal wires spaced 13 mm apart. A plastic foot rest of 25 cm × 40 cm (17 mm wide slats separated by 12 mm wide slits) was mounted onto the wire floor in the middle of the cage.

The semi-group pens (Van der Vinne, Brucht, the Netherlands) each housed 4 does and measured 100 cm × 200 cm (Fig. 1). In addition, a plastic slatted platform of 200 cm × 30 cm was available to the does. Three days prior to each kindling three walls were added, separating each pen into 4 equal units of 50 cm × 100 cm plus 1500 cm^2 of platform. As such, the space allowance per doe was greater in these semi-group pens than in the individual cages (6500 vs. 5092 cm^2 per doe, including platforms). Furthermore, the nest box (33 cm × 24 cm × 28 cm length × width × height) was external in the semi-group pens, thus not taking up any pen space. Pens were roofless and the only height limitation in these systems occurred underneath the platform mounted 30 cm above floor level. Eighteen days post-kindling the three walls were removed again and all four does and their litters could use the entire pen area, but does were barred from access to the nest-boxes to provide a safe haven for the kits to flee to in case they were attacked by the does. The wire floor semi-group pens were equipped with the same floor and footrest as the single-doe cages (one 1000 cm^2 footrest per doe). The plastic floor of the other semi-group pens had 13 mm wide slats separated by 12 mm wide slits. These different floor types were included in the experiment mainly to study their influence on foot health (Buijs et al., 2014) and (re)production (Maertens and Buijs, 2013).

This experiment was a systems comparison: not only did the social situation differ between the semi-group pens and single-doe housing, the amount of space per animal also differed. This confounding between housing system and space allowance was made intentionally, as a successful (semi-)group housing system is likely to require more space for rabbits to avoid each other in times of conflict. The single-doe system used as a control treatment was a modern, commercial system that is commonly applied in Western Europe.

2.3. Data collection

2.3.1. Behavioural analysis

During the second cycle 16 single-doe cages and eight semi-group pens (four per floor type) were video recorded for behavioural analysis. Thirty minute video recordings were made immediately subsequent to mixing (around mid-day) and 12 h thereafter (around midnight). 3.5 and 4 days later the pens were recorded again for another 30 min (to which we will refer as midnight and mid-day 4 days after mixing in the rest of this article). The last set of 30-min recordings was made 12 and 12.5 days after mixing (to which we will refer as mid-day and midnight 12 days after mixing). Behaviour was analysed by one observer using continuous recording in Observer 8.0 (Noldus information technology, Wageningen, the Netherlands). All does in the observed pens and cages were used as focal animals. The behaviour of the kits was not analysed. As the behaviour of animals housed in the same pen is interdependent, our analysis was based on the mean of the observations on the four does in each semi-group pen. For the sake of similarity we followed the same procedure for does in single-doe cages (averaging the results of four adjacent cages). The results are presented as a percentage of the total observation time. In addition to a group of mutually exclusive behaviours (Table 2), platform use and bodily contact were scored as non-mutually exclusive categories (e.g. a doe could be lying in full recumbency, on the platform, whilst in bodily contact).

2.3.2. Wound scoring

The number and severity of wounds per doe were determined directly before mixing and again 4 days later. All does from semi-group housing were scored in each round. Wound scoring was only performed for does in semi-group pens as those from single-doe cages were not expected to become wounded. This expectation was checked in the last cycle of the experiment. Wounds were scored according to Rommers et al. (2014): score 1: superficial and <1 cm, score 2: superficial ≥1 cm or deep and <1 cm, score 3: deep and ≥1 cm. The number of wounds before mixing was subtracted from the number of wounds after mixing (per class) and the difference was used to allocate does to wound categories: (0) no extra wounds, (1) 1–4 extra class 1 wounds, (2) 1–4 extra class 2 wounds or ≥5 extra class 1 wounds, (3) ≥1 extra class 3 wounds or ≥5 extra class 2 wounds.

2.3.3. Weight loss

All does were weighed on day 18 and 30 of lactation to determine weight loss in the interval between grouping and weaning.

2.3.4. Adrenal weight

At the end of cycle four all does were euthanized by an intravenous injection of T-61® (Intervet International, Boxmeer, the Netherlands) preceded by anaesthesia with an intramuscular

Table 2
Ethogram used for behavioural analysis.

Behaviour	Description
Stationary	Sitting, standing or lying (excluding lateral or stretched out lying) including movement without hind leg displacement
Full recumbency	Lying laterally or stretched out (both hind legs protruding at the side or back of the body)
Locomotion	Hopping or running without being chased or displaced
Flee/retreat	Hopping or running whilst being chased or in reaction to approach by another rabbit
Attack/chase	Fighting (biting, scratching, pinning), charging or chasing another doe
Doe-directed sniff/groom	Directing the nose towards another doe or manipulating it gently with the snout
Kit-directed sniff/groom	As above, except directed at a kit
Groom	Moving the mouth or paw over the own fur
Comfort behaviour	Yawning and stretching
Rear	Standing up on the hind legs
Ingestive	Eating or drinking
Other	Behaviours other than described above
Not seen	
Platform use	Being present on the platform (regardless of which type of behaviour was displayed there)
Bodily contact	Body–body physical contact with another doe

injection of xylazine (Xyl-M 2%®, VMD, Arendonk, Belgium) and ketamine (Anesketin®, Eurovet, Heusden-Zolder, Belgium). Subsequently both adrenal glands were collected and weighed on a precision balance (Sartorius CP324S).

2.4. Statistics

Behaviour was analysed separately for each of the 6 timeslots (mid-day and midnight 0, 4 and 12 days after mixing) using Kruskal–Wallis tests in R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria). Mean values of four does (either from the same semi-group pen or from four adjacent single-doe cages) expressed as a percentage of the observation time were used as input for this analysis. Floor type effects were evaluated within the semi-group systems. As such effects were scarce, semi-group systems were treated as one category when assessing the differences between single-doe and semi-group housing.

Weight loss and adrenal weight were analysed using linear mixed models in SAS 9.4 (SAS Institute, Cary, USA). First, we evaluated floor type effects within the semi-group systems. For weight loss the model included floor type (plastic slats vs. wire), cycle and floor type × cycle as fixed factors and doe and group (i.e. the four does housed in the same semi-group pen or four does housed in adjacent single-doe cages) as random factors. For adrenal weight floor type, side (i.e. left or right adrenal) and floor type × side were used as fixed factors and doe as random factor. After confirmation that floor type did not affect weight loss or adrenal weight, data from semi-group housing on wire and semi-group housing on plastic slats were pooled into one category to assess the effects of housing (single-doe vs. semi-group) on adrenal weight and weight loss. Other than that the factor “floor type” was replaced by the factor “housing”, the models were the same as described above. Wound scores of semi-group housed does were analysed using a cumulative logit model in SAS 9.4. Floor type (wire vs. plastic), cycle and floor type × cycle were included as fixed factors and doe and group as random factors.

3. Results

3.1. Behaviour

In single-doe housing no agonistic behaviour (attacking/chasing and fleeing/retreating) was observed and doe directed sniffing/grooming was extremely rare (Table 3), even though at least elements of such behaviours were possible through the wire cage walls.

Few effects of floor type were observed and when such effects occurred they were unique to a time slot. In the first 30 min following mixing the percentage of time spent on the platform differed between semi-group systems with different floor types (median + interquartile range plastic: 0.9% (0.7–1.5), wire: 5.0% (3.1–8.1), $P = 0.04$), as did the percentage of time spent in bodily contact (plastic: 4.0% (2.0–6.1), wire: 0.6% (0.3–1.1), $P = 0.04$). During mid-day 4 days after mixing the time spent stationary in the semi-group systems differed between floor types (plastic: 73% (71–77), wire: 87% (85–89)) and the time spent in full recumbency tended to do so (plastic: 12% (9–16), wire: 3% (0–6), $P = 0.08$). No other differences in behaviour occurred between the floor types in any of the time slots ($P > 0.10$). As floor type effect was scarce, data from both types of semi-group system were treated as one category when analysing the differences between single-doe housing and semi-group housing.

In the first 30 min following mixing, semi-group does spent a greater percentage of time stationary, in locomotion, fleeing/retreating, attacking/chasing, sniffing other does and kits and rearing than single-housed does did (Table 3). They spent a smaller percentage of time in full recumbency, ingesting and in bodily contact, and tended to spend a smaller percentage of time grooming. The following night the differences in kit-directed sniffing and ingestive behaviour were no longer significant, but the other differences between single-doe housing and semi-group housing remained, although effect sizes were usually smaller than directly after mixing (especially for agonistic behaviour).

Four days after mixing the number of behaviours for which the percentage of time differed between single-doe cages and semi-group pens was reduced even further. However, around midnight semi-group does still spent significantly greater percent of time on locomotion, agonistic behaviour and sniffing/grooming other does and kits than does in single-doe housing. At mid-day semi-group does spent greater percent of time stationary, tended to spend more time sniffing/grooming other does and spent less time grooming. The differences between the systems were generally smaller than on the day and night directly following mixing.

At mid-day 12 days after mixing does in semi-group housing spent a greater percentage of time on lying full recumbency and sniffing/grooming other does, and tended to spend a greater percentage of time on locomotion than does in single-doe housing. Semi-group does spent (or tended to spend) a greater percentage of their midnight time on locomotion, agonistic behaviour, sniffing other does, and rearing than does in single-doe housing.

Compared to does from single-doe housing, does from semi-group housing spent (or tended to spend) a smaller percentage of time on the platform at mid-day and midnight 4 days after mixing, but spent a greater percentage of time on the platform at midnight 12 days after mixing.

3.2. Wounds

As expected, none of the does in single-doe housing had wounds when they were checked at the end of the last cycle. In the average cycle, 78% of all does in semi-group housing received at least one new wound during the 4 days following mixing (Table 4). A weak tendency for a floor type effect was detected ($F_{1,82} = 2.8$, $P = 0.098$):

Table 3
Time spent on different behaviours as a percentage of total observation time (medians + interquartile range). Note that on day 4 only, nighttime observations were made prior to daytime observations. Kruskal–Wallis analyses were performed using mean values per semi-group pen (8 pens in total) or per cluster of single doe cages (4 clusters) as input.

	Day			Night		
	Single-doe	Semi-group		Single-doe	Semi-group	
Day of mixing						
Stationary	70.4 (69.2–71.2)	79.3 (78.3–82.3)	**	43.5 (38.54–45.8)	67.9 (57.2–72.5)	**
Full recumbency	11.88 (10.44–13.4)	0.29 (0–1.03)	**	26.68 (21.85–30.58)	0.65 (0–2.99)	**
Locomotion	0.69 (0.59–0.76)	4.34 (3.77–4.99)	**	0.37 (0.25–0.54)	3.01 (2.45–3.31)	**
Flee/retreat	0 (0–0)	1.96 (1.11–2.45)	**	0 (0–0)	0.49 (0.25–0.89)	**
Attack/chase	0 (0–0)	5.27 (4.38–8.42)	**	0 (0–0)	0.24 (0.16–0.45)	**
Doe-directed sniff/groom	0 (0–0)	1.35 (1.17–2.05)	**	0 (0–0.01)	0.24 (0.11–0.9)	*
Kit-directed sniff/groom	0.05 (0.02–0.1)	0.32 (0.21–0.76)	*	0.08 (0.05–0.13)	0.33 (0.14–0.49)	ns
Groom	3.24 (1.65–4.98)	0.94 (0.54–1.86)	#	14.22 (11.75–15.96)	2.74 (1.84–4.51)	**
Comfort	0 (0–0)	0 (0–0.05)	ns	0 (0–0.01)	0 (0–0)	ns
Rear	0 (0–0.02)	0.9 (0.6–1.0)	**	0 (0–0.02)	0.28 (0.15–0.47)	*
Ingestive	12.42 (10.66–14.41)	0.12 (0.06–0.87)	**	11.96 (9.31–13.65)	7.82 (6.42–10.63)	ns
Not seen	1.16 (0–2.33)	0 (0–1.18)	ns	7.98 (5.36–10.98)	8.91 (5.79–17.24)	ns
Platform use	0.94 (0.40–3.69)	2.81 (0.95–4.11)	ns	11.32 (0.09–24.17)	19.62 (3.97–25.01)	ns
Bodily contact	11.8 (10.64–15.16)	1.6 (0.75–3.09)	**	11.03 (8.94–14)	0.6 (0–3.04)	*
4 Days after mixing						
Stationary	64.6 (62.0–67.5)	82.2 (73.6–86.0)	ns	53.4 (48.3–60.2)	50.6 (47.5–66.0)	ns
Full recumbency	11.55 (8.14–16.19)	5.94 (3.03–11.04)	ns	8.88 (7.09–13.68)	7.93 (2.95–12.99)	ns
Locomotion	0.33 (0.23–0.45)	0.47 (0.41–0.63)	*	0.59 (0.51–0.97)	2.36 (1.61–2.87)	*
Flee/retreat	0 (0–0)	0 (0–0)	**	0 (0–0)	0.28 (0.21–0.32)	**
Attack/chase	0 (0–0)	0 (0–0)	*	0 (0–0)	0.06 (0.04–0.11)	*
Doe-directed sniff/groom	0 (0–0)	0.06 (0–0.22)	**	0 (0–0)	0.40 (0.2–0.55)	**
Kit-directed sniff/groom	0.09 (0.06–0.76)	0.03 (0–0.1)	*	0.04 (0–0.1)	0.38 (0.09–0.74)	*
Groom	7.46 (5.72–9.41)	3.78 (2.1–4.35)	ns	10.65 (7.98–13.82)	8.41 (4.74–13.56)	ns
Comfort	0.02 (0.01–0.04)	0.04 (0.03–0.07)	ns	0 (0–0.07)	0 (0–0.03)	ns
Rear	0 (0–0)	0 (0–0.01)	ns	0.08 (0.05–0.15)	0.71 (0.07–1.01)	ns
Ingestive	9.51 (6.96–10.73)	7.84 (4.45–9.59)	ns	13.73 (9.08–18.89)	16.22 (8.66–17.8)	ns
Not seen	0.19 (0–4.78)	0 (0–0)	*	5.75 (3.3–8.73)	6.94 (0.34–19.04)	ns
Platform use	15.87 (14–19.42)	0 (0–5.83)	#	29.47 (20–40.12)	15.56 (3.83–20.43)	*
Bodily contact	2.87 (0.6–7.62)	5.58 (4.47–8.34)	ns	5.76 (2.33–9.07)	2.78 (1.03–3.78)	ns
12 Days after mixing						
Stationary	75.1 (73.0–75.8)	74.4 (64.9–81.7)	ns	60.8 (55.2–67.3)	48.8 (46.5–64.2)	ns
Full recumbency	0 (0–0.89)	9.22 (4.41–16.91)	**	7.29 (4.37–9.01)	10.3 (8.56–13.09)	ns
Locomotion	0.13 (0.11–0.14)	0.28 (0.17–0.35)	#	0.19 (0.17–0.25)	0.78 (0.61–1.41)	*
Flee/retreat	0 (0–0)	0.01 (0–0.01)	ns	0 (0–0)	0.08 (0.03–0.17)	*
Attack/chase	0 (0–0)	0 (0–0)	ns	0 (0–0)	0.01 (0–0.03)	#
Doe-directed sniff/groom	0 (0–0)	0.06 (0.02–0.14)	*	0 (0–0)	0.42 (0.35–0.64)	**
Kit-directed sniff/groom	0.19 (0–0.4)	0.05 (0–0.14)	ns	0.05 (0.02–0.11)	0.33 (0.08–0.64)	ns
Groom	11.97 (7.23–16.25)	7.82 (4.28–8.70)	ns	13.62 (11.25–16.41)	13.39 (11.66–17.28)	ns
Comfort	0.03 (0–0.08)	0.07 (0–0.10)	ns	0.01 (0–0.02)	0.06 (0.01–0.11)	ns
Rear	0 (0–0.01)	0.01 (0–0.08)	ns	0.08 (0–0.18)	0.65 (0.2–1.61)	#
Ingestive	5.98 (5.21–7.17)	5.56 (3.44–6.73)	ns	9.01 (7.62–10.27)	10.87 (6.61–14.19)	ns
Not seen	1.52 (0–6.61)	0 (0–0)	*	5.72 (2.43–12.21)	1.9 (0.10–6.12)	ns
Platform use	0.06 (0–0.45)	0 (0–5.90)	ns	4.32 (2.72–6.12)	28.2 (24.31–33.20)	*
Bodily contact	0 (0–1.78)	3.47 (0.62–4.90)	ns	0 (0–1.67)	1.89 (0–7.72)	ns

** $P < 0.01$.
* $P < 0.05$.
# $P < 0.10$.

the odds of being in a higher wound category were 2.08 times as high for does housed on the wire floor as for does housed on the plastic floor. The odds of being more severely wounded did not differ significantly between the cycles ($F_{3,82} = 0.82$, $P = 0.49$) and no significant floor type × cycle interaction was found ($F_{3,32.83} = 1.3$, $P = 0.29$).

3.3. Weight loss

Weight loss did not differ between semi-group does housed on plastic slats and semi-group does housed on wire (floor type × cycle: $F_{3,143} = 0.3$, $P = 0.85$; floor type: $F_{1,60.2} = 0.13$, $P = 0.72$). Therefore, data from both types of semi-group system were treated as one category when analysing the differences between single-doe housing and semi-group housing. Weight loss between 18 and 30 days of lactation was influenced by a housing × cycle interaction. During the first experimental cycle only, semi-group does lost more weight than does from single-doe housing. Weight loss increased with increasing cycle number in both housing systems, this change being more incremental for single-doe housing (Table 5).

3.4. Adrenal weight

Adrenal weights of semi-group does housed on plastic slats and semi-group does housed on wire did not differ (floor type × side: $F_{1,38.9} = 1.7$, $P = 0.20$; floor type: $F_{1,41.4} = 1.2$, $P = 0.29$) and were therefore pooled when analysing differences between single-doe housing and semi-group housing. Adrenal weight was influenced by a housing × side interaction ($F_{1,60.7} = 7.5$, $P = 0.008$). The right adrenal gland was 13% lighter than the left adrenal gland for rabbits in single-doe housing only (Table 5). No main differences between single-doe housing and semi-group housing were observed ($P > 0.10$).

Table 4
Raw percentage of does in the four wound categories: (0) no extra wounds, (1) 1–4 extra class 1 wounds, (2) 1–4 extra class 2 wounds or ≥5 extra class 1 wounds, (3) ≥1 extra class 3 wounds or ≥5 extra class 2 wounds. Twenty-four does were scored per floor type per reproductive cycle included in the experiment.

Cycle	Wound category	Semi-group – plastic	Semi-group – wire
1	0	33.3	12.5
	1	37.5	12.5
	2	20.8	37.5
	3	8.3	37.5
2	0	20.8	20.8
	1	20.8	16.7
	2	41.7	29.2
	3	16.7	33.3
3	0	33.3	20.8
	1	16.7	16.7
	2	33.3	33.3
	3	16.7	29.2
4	0	8.3	25.0
	1	54.2	45.8
	2	37.5	20.8
	3	0.0	8.3

Table 5
Weight loss between day 18 and day 30 of the four reproductive cycles included in the experiment (corresponding with the 2nd to 5th reproductive cycle in the does life, as does had kindled once prior to the experiment), and post-experimental adrenal weight (LSMEANS ± SEM). Twenty-four does from single-doe cages and 48 from semi-group pens were included. **Difference between single-doe and semi-group housing ($P < 0.01$). Within a column and indicator, means lacking a common superscript letter differ significantly.

	Single-doe	Semi-group
Weight loss (g)		
Cycle 1	10 ± 46^{c}	$192 \pm 33^{b,**}$
Cycle 2	66 ± 46^{c}	154 ± 33^{b}
Cycle 3	178 ± 47^{b}	190 ± 33^{b}
Cycle 4	407 ± 47^{a}	337 ± 33^{a}
Adrenal weight (g)		
Left	0.214 ± 0.011^{x}	0.203 ± 0.008^{x}
Right	0.187 ± 0.011^{y}	0.199 ± 0.008^{x}

4. Discussion

4.1. Locomotion and social behaviour

Semi-group housing was hypothesized to (temporarily) alleviate spatial restrictions on locomotion. In line with such restrictions, single-housed does spent very little time on locomotion (0–1%, confirming previous research (Alfonso-Carrillo et al., 2014; Hansen and Berthelsen, 2000)). Semi-group does spent a greater percentage of time on locomotion, but not much greater (4% directly after mixing, 3% 12 h later, 2–1% at midnight 4 and 12 days later). This rather low level of locomotion in group systems also confirms previous research (Rommers et al., 2014). Rabbits are sometimes claimed to be more reliably active at dusk than during the night (e.g. Villafuerte et al., 1993), which could mean that we missed the most active phase by observing at midnight. But this seems unlikely as most studies of the circadian rhythms in rabbits suggest activity throughout the night (Sato et al., 1995; Eisermann, 1988; Kennedy et al., 1994).

The post-mixing locomotion peak could be interpreted as a rebound effect (in which a highly motivated behaviour restricted by external factors will be performed at a high frequency once the restricting factors are removed (Nicol, 1987)). However, this seems unlikely because pet rabbits do not display rebound locomotion when moved to a larger pen (Dixon et al., 2010). Moreover, the 4% increase is much lower than what has previously been claimed as evidence of locomotion rebound in other species (14–50%: Dellmeier et al., 1990; Rushen and de Passille, 2014; Loberg et al., 2004; Houpt et al., 2001; Mal et al., 1991). In most rebound studies animals mainly displayed intense types of locomotion (running/trotting/galloping instead of walking), whereas we rarely observed running without being chased or chasing. Instead, the post-mixing locomotion peak may have been due to exploration of the expanded housing and new pen mates. The possibility that does are actually highly motivated to show a small amount of extra locomotion (and that the slightly greater percentage of time spent on locomotion in the semi-group pens thus did have a positive impact on welfare) can not be fully discounted, however. Even behaviours that take up a small percentage of the time-budget can be strongly motivated (e.g. nursing takes up 0.2% of does' time budget, but barring them from nursing will lead to a clear frustration response). To determine if this is also true for locomotion, additional (motivation or preference) tests will be needed. Specific layout choices may also have affected locomotion, or does may have felt unsafe to show locomotion because it could attract attention of other (aggressive) does.

(Semi-)group housing allows does to have physical interactions that are impossible in single-doe cages. However, the percentage of time spent on physically interaction with adults in a manner assumed to be positive (grooming or sniffing them) was very low in our semi-group system (1% post-mixing and less in later timeslots). Again, low levels of physical interaction does not necessarily mean that this behaviour is unimportant. But without data on does' motivation for a limited amount of physical contact, it is not conclusive evidence that it is important either. Post-mixing bodily contact was less common in semi-group pens than in single-doe cages. This may be because does were still unacquainted and thus reluctant to make contact without the safety of a wall between them. Although this difference had disappeared 4 days after mixing, the percentage of time semi-group does spent in bodily contact never exceeded that in single-doe housing.

To summarize, the positive effects of our semi-group system on behaviour seemed small and occurred mainly shortly after mixing. Although we assumed all locomotion and non-agonistic social interactions to be positive in nature, this may not always have been the case. For instance, increased locomotion and social interaction may represent attempts to regain safety by exploring the novel pen area and social partners. Also, although we discerned between spontaneous locomotion and withdrawal based on the behaviour of the focal's pen mates, subtle signals causing does to withdraw may have been overlooked. As such, even the low levels of positive behaviour may have been an overestimation.

4.2. Agonistic, ingestive and resting behaviour and wound scores

One adverse effect of grouping is that this will lead to a period of acquaintance, often characterized by agonistic behaviour and unrest. We found that directly after mixing, does in semi-group housing spent 5% of their time on attacking/chasing other does and another 2% on fleeing/retreating from them. They almost completely stopped feeding (0.1%), whereas feeding was quite common in single-doe housing in this timeslot (12%). Such differences receded quickly, less than 1% of the time-budget was spent on agonistic interactions from 12 h after mixing on, and ingestive behaviour no longer differed between the systems at that time. Still, a considerable percentage of moderately (category 1 or 2: 58%) and severely (category 3: 20%) wounded does were found in the semi-group pens, likely as a result of the observed agonistic interactions. These percentages exceed those of previous studies on (semi-)group housing: 33–43% category 1 or 2 does and 1–9% category 3 does (Andrist et al., 2013; Rommers et al., 2014). This may be due to differences in pen layout, genetics or interpretation of the wound detection protocol (rabbits' thick fur makes their wounds hard to detect).

Compared to does in single-doe cages, semi-group does spent less time fully recumbent on the day and night following mixing, suggesting that they were less at ease. The opposite occurred on day 12 when semi-group does spent more time fully recumbent during mid-day (i.e. in the resting phase). This may be due to increased disturbance of single-housed does by their kits as these grew and took up more space (which would have less impact in the semi-group system where space allocation was more liberal).

To summarize, our semi-group system had several unwanted effects on behaviour shortly after mixing and led to considerable wounding. It is possible that we would have found less adverse effects of the semi-group system if we had grouped does with familiar conspecifics, as such effects are known in sows (Arey, 1999). However, we chose not to do so, as groups are unlikely to remain stable in commercial practice as does that do not become pregnant upon insemination are moved to different production groups.

4.3. Platform use

Platform use in single-doe cages varied greatly (from 0 to 29% depending on the time slot). It was lower than reported by others who used 24 h behaviour sampling (Miko et al., 2014: 32%; Alfonso-Carrillo et al., 2014: 25%). This may be due to our observation times, as platform use has been reported to be most prominent between 16.00 and 22.00 h (Alfonso-Carrillo et al., 2014). Platform use in single-doe housing was especially low 12 days after mixing. At this time, kits often used the platforms in the single-doe cages, but not in the semi-group pens. The greater use of the platform by semi-group does may be because they were more successful in using the platforms to get away from their kits. Why the kits used the platform less in semi-group housing is unclear, as platform height was similar in both systems. It may be that semi-group kits had more to explore on the ground level and were therefore less interested in the platform.

4.4. Adrenal weight and body weight loss

The lack of a difference in adrenal weight between single-doe and semi-group housing confirms studies on social housing of laboratory rabbits (Noller et al., 2013; Whary et al., 1993). Post-mortem adrenal weight may not be fully informative of rabbits' glucocorticoid status throughout grouping however. For instance, Noller et al. (2013) found higher mid-experiment blood cortisol levels in unstable than in stable groups, but no differences in post-mortem adrenal weight. Thus, we may not have found differences due to the indicator we chose. It seems unlikely that an increase in adrenal gland size due to social stress was countered by a decrease in gland size due to the more liberal space allowance of our semi-group system, as spatial restriction does not affect glucocorticoid levels in sub-adult rabbits (Buijs et al., 2011).

Semi-group housing led to increased weight loss during experimental cycle one only, possibly due to chronic stress, increased behavioural activity or a reluctance to feed. The disappearance of this effect by the second experimental cycle (when behavioural observations were conducted) impedes an evaluation of the possible causes based on behaviour. It also means that weight loss due to any of these causes is unlikely to be an important concern in semi-group housing, at least not from the second cycle onwards.

5. Conclusions

Our semi-group pens increased some behaviours assumed to reflect improved welfare, but also caused unwanted behaviour and wounding. Stress indicators showed little effect of semi-group housing. In future, different methods may be applied to see how rabbits evaluate the pros and cons of semi-group systems themselves (e.g. preference or motivation testing). However, based on the present study we found no clear evidence of major behavioural changes that could outweigh the high percentage of wounded does in our semi-group system in terms of overall welfare. This may be partly due to the specific system and management we used, aiming to stay close to commercial practice to allow quick implementation if successful. For instance, it is certainly possible that semi-group systems impact more positively on welfare if groups are kept stable throughout the cycles, or if improvements in pen lay-out would facilitate locomotor behaviour further.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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