

EFFECT OF GROUP SIZE AND ESCAPE ENRICHMENT ON REPRODUCTIVE PERFORMANCE OF BREEDING DOES IN PART-TIME GROUP HOUSING

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Abstract: Societal demands for group housing of social farm animals such as rabbits are increasing due to animal welfare concerns. When breeding does are housed in groups, maternal protective behaviour negatively affects the reproductive performance of the does. In part-time group housing, does are housed in single-litter cages starting shortly before parturition until the first part of lactation and then group housed for the second part after their protective behaviour has diminished and the kits are more resilient. The aim of this study was to evaluate the reproductive performance of breeding does in a part-time group housing system with provision of escape enrichment and different group size (and concomitant different stocking density, as pen size remained unchanged). For each of the four consecutive reproductive cycles, 42 does with their kits switched at 22 d post-partum (pp) from single-litter to group housing for a total period of 13 d in pens of 200×102 cm by removing walls between four adjacent single-litter cages. Newly created group pens (N=12 pens per treatment) varied in group size, stocking density and enrichment: groups of either three or four does were divided over same-size pens with or without escape enrichment (2×2 factorial design). The escape enrichment was comprised of two sets of PVC pipes and two extra elevated platforms. Does were weighed and kits counted at parturition, after which kits were cross-fostered. Kits were counted and weighed again 22 and 35 d pp. Results for mean doe fertility (90.0%), number of kits (9.1 kits/litter) and kit weight (1037.3 g/kit) at weaning were comparable with professional farms using single-litter cages. No significant effects of group size and escape enrichment were found for any of the reproductive parameters. While housed in group, seven does were removed from the experiment, from which four does were severely injured. Post-grouping kit mortality was rather low (6.7%), but three kits were euthanised due to severe injuries. In conclusion, provision of escape enrichment and altered group size (and stocking density) had no profound effect on the reproductive performance of part-time group housed does.

Key Words: part-time group housing, reproductive performance, animal welfare, rabbits, breeding does, kits.

INTRODUCTION

Individual housing of social farm animals such as rabbits is subject to increasing societal criticism, as group housing is perceived to be more animal-friendly. After submission of the 'End the Cage Age' initiative in 2020, the European Commission committed to provide a legislative proposal to phase out, and eventually prohibit, the use of cage systems for farm animals, including rabbits (DG Health and Food Safety, 2021). In the Netherlands, Germany, Switzerland

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and Belgium, multiple-litter group pens are already used when rearing weaned meat rabbits (DG Health and Food Safety, 2017). In contrast, breeding and nursing does are most often kept in single-litter cages in which the rabbits cannot sufficiently express natural behaviours such as running and jumping and bodily social contact between does. Single-litter cages can also become overcrowded as the kits grow. Housing multiple does with their kits in group pens has been explored as an alternative (Rommers *et al.*, 2006; Andrist *et al.*, 2013). Benefits of group housing include increased absolute available space per animal and social contact among does. The main drawback is maternal protective behaviour, which results in aggression (and consequently injuries), stress and associated negative effects on reproductive performance of the breeding does (Mugnai *et al.*, 2009; Szendrő *et al.*, 2013; Machado *et al.*, 2019).

In part-time group housing systems, does are housed in single-litter cages starting just before parturition until the first part of lactation, the period in which they are most likely to express aggressive behaviour towards other does (Buijs *et al.*, 2014). Starting in the second part of the lactation period, when the kits are more resilient and protective doe behaviour has decreased (Rödel *et al.*, 2007), does are housed in groups for the remainder of the reproduction cycle (Maertens and Buijs, 2013). Interest in this housing system is growing, as it provides reproductive performance similar to that in single-litter housing (Maertens and De Bie, 2017; Dal Bosco *et al.*, 2019; Machado *et al.*, 2006; Munari *et al.*, 2020).

In Belgium, new legislation requiring group housing of breeding does on commercial rabbit farms will take effect in 2025 (Royal Decree 29 June 2014), although specific guidelines have not yet been established. The Flemish government therefore commissioned a research project to provide practical recommendations regarding group housing of breeding does during at least some part of their reproductive cycle. Part of this project included research on the effect of the timing of grouping on reproduction and welfare (Van Damme *et al.*, 2022). Data from that study on the number and severity of skin injuries indicated that grouping of does with their kits seemed more desirable at 22 in comparison to 25 and 28 d post-partum (pp).

Previous research indicated that design of the group pen and occupation rate may be potentially promising strategies for optimising part-time group housing systems. Buijs *et al.* (2016) studied group sizes of four and eight does when the kits were 18 d old. The authors reported a higher frequency of offensive agonistic behaviour in the larger groups. In the groups of four does, a decrease in defensive agonistic behaviour over time was observed after grouping, whereas this trend was absent in the larger groups. Furthermore, the authors found a trend showing more offensive agonistic behaviour in the larger groups of eight does compared with the smaller groups. In the study of Zomeño *et al.* (2017), either two or four single-litter cages were joined together by opening hatches, allowing the does to pass through the cages. In groups of four connected cages (four does), more boxing and chasing behaviour was observed compared with groups of two connected cages. The provision of two hatches instead of one between the cages resulted in higher rates of attacking, chasing and mounting behaviour compared with cages connected with one hatch. The authors posited that the additional hatch resulted in postponed confrontation and thus increased aggression. In contrast, Rommers *et al.* (2013), observed that the provision of hiding places slightly reduced aggression in groups of four does.

In accordance with the commissioned Flemish research project, the present aim was to study the effect of group size and pen design on the reproductive performance of breeding does housed in group with their kits from 22 d pp onwards. Group size and escape enrichment were tested in combination as the effect of escape possibilities may be influenced by group size and vice versa. As the dimensions of the group pens remained constant regardless of the number of does, a change in group size also entailed a change in stocking density, making it impossible to disentangle the possible effect of both factors. In the same trial, doe behaviour and skin injuries of both the does and kits were monitored during group housing (Van Damme *et al.*, 2023, unpublished), but will not be discussed in this paper.

MATERIALS AND METHODS

All protocols and procedures were approved by the Ethics Committee for the Use of Animals in Research (EC 2020/378) of Flanders Research Institute for Agriculture, Fisheries and Food (ILVO).

Animals and housing

For the trial, 66 nulliparous 16-wk-old does (female breeding rabbits) were purchased from Hycole (Marcoing, France) and transported to Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Melle, Belgium). On arrival, 42 does were randomly chosen and divided into type A single-litter cages (50×102 cm with a plastic slatted floor, open roof and elevated platform of 50×30 cm, Figure 1A). The remaining 24 does were housed in type B single-litter cages (38×103×63 cm, wire slatted floor with a plastic comfort mat of 25×40 cm and elevated platform of 38×28 cm, Figure 1B). Both types of cages were equipped with a wooden gnawing block, a feeder and water nipple.

At the age of 19 wk, all does were artificially inseminated (Al) for the first time. One week before the expected birth of the first litter, does were provided a nest box ($34 \times 24 \times 28$ cm in type A cages and $38 \times 23 \times 28$ cm in type B cages) and nesting material (flax and wood shavings). After birth of the first litter, does again received Al at 17 d pp.

At 35 d pp, does and their cross-fostered litters were transferred to a cleaned and disinfected compartment until 39 d pp. After this, the does were returned to the previous compartment to prepare for their next litter. From this point onwards (39 d pp), kits were housed in group pens with multiple litters until slaughter age (10-11 wk). This whole process was repeated a total of five times, resulting in five consecutive reproductive cycles of 49 d each (Al at 17 d pp and a gestation period of 32 d). This form of intensive reproduction means that for the majority of the reproduction cycle, does nurse their young while they are pregnant with their next litter. After every parturition, liveborn kits were cross-fostered based on their size to create homogenous nests. In the first reproduction cycle, each doe was assigned 9 kits, but this rose to 10 in the other cycles. The surplus liveborn kits were euthanised 1 d pp.

All animals had unlimited access to fresh water and fresh hay was provided in all cages during the first three weeks of each cycle. Pregnant and/or lactating does were given commercial pellet rabbit feed (Country's Best CUNI TOP pure, Versele-Laga, Deinze, Belgium) *ad libitum*. Non-pregnant does with no kits to nurse were restricted to 150 g of feed per day. Three days prior to weaning until one week before slaughter, meat rabbits were fed the same feed *ad libitum* with an added coccidiostat (Country's Best CUNI TOP plus, Versele-Laga, Deinze, Belgium). In the second



Figure 1: A: single-litter cage (type A), 50×102 cm with a plastic slatted floor, open roof and elevated platform of 50×30 cm. B: single-litter cage (type B), $38 \times 103 \times 63$ cm, wire slatted floor with a plastic comfort mat of 25×40 cm and elevated platform of 38×28 cm.

reproduction cycle, one week after parturition, all does received a *Pasteurella* vaccine. Artificial light was provided at a cycle of 12 L:12 D, except for 7 d prior to Al when the light cycle was changed to 16 L:8 D. Air temperature was set at 20-21°C and relative air humidity was between 60 and 75%.

At the end of the trial and after nursing their last litters, does were put up for adoption (in cooperation with accredited Belgian shelters) for permanent rehoming. Kits were sold as meat rabbits to a slaughterhouse.

Experimental design and treatments

The effect of escape enrichment and group size and their interaction on the reproductive performance of breeding does in a part-time group housing system was evaluated. During the first four reproduction cycles, groups of either three or four does with their litters were created at 22 d pp and housed in group pens (N=42 does per cycle). Pens were assigned one of the following treatments in a completely randomised block design (N=3 pens per treatment and cycle): group of four does with no additional enrichment (4N), four does with additional environmental enrichment (4Y), three does with no additional enrichment (3N) and three does with additional environmental enrichment (3Y). The additional enrichment was added just before group housing began. It was comprised of two sets of PVC pipes mounted underneath the platform (1 large pipe and 2 small pipes with a diameter of 20 and 9 cm respectively, arranged in a pyramid structure with the larger pipe at the top) and two smaller elevated platforms mounted in the back corners of the pens 30 cm above the first level platform (Figure 2). Groups of three or four does with their litters were created by merging four adjacent type A single-litter cages. In group pens of three does, the fourth cage was empty (Figure 2). As such, for every cycle four type A single-litter cages were merged regardless of whether all cages were occupied by a doe and her litter. The absolute available surface thus remained identical for each treatment,



Figure 2: Group pens, created 22 d post-partum from four adjacent (type A) single-litter cages by removing three wire walls (dotted lines). Figure represents group housing of three does with escape enrichment (fourth cage is empty, kits not shown in figure). Escape enrichment entails 2 sets of PVC pipes (1 large pipe and 2 small pipes with a diameter of 20 and 9 cm respectively) and 2 sets of smaller elevated platforms mounted in the back right and left corner of the pen above the first level platform. Three panels underneath the first level platform (structural supports) and feeders at the front of the pen (flat cubes in figure) were present in all pens. Water nipples were located next to the feeders (not shown).

but group size (and accordingly stocking density of does and kits) differed. Group housing lasted for 13 d and ended 35 d pp, after which does and kits were transferred to a new compartment.

Treatments were repeated during the first four reproduction cycles (August 2020 until February 2021). Pens were never given the same treatment more than once to account for possible location/pen effects. Between reproduction cycles, does were assigned to a different pen, different treatment and either three or two unfamiliar does. Non-pregnant, sick, injured or deceased does were replaced with pregnant does from type B single-litter cages in between reproduction cycles but not during the group housing phase (day 22 until 35 pp).

Data collection

After each parturition, does were weighed individually 1 d pp in all reproduction cycles. On the same day, the number of liveborn and stillborn kits per doe was recorded and the success rate of Al (in the previous cycle) could be confirmed. Litter sizes were counted and kits were weighed individually at 22 and 35 d pp. On day 22 pp, all does and kits were marked with coloured paint for individual identification. Mortality of both does and kits (after cross fostering) was registered, including the date and suspected cause of death (if known).

Statistical analysis

All analyses were conducted with the statistical software R 4.1.2 (The R Foundation for Statistical Computing). Data was assumed to be sufficiently normally distributed based on visual inspection of the residuals of the models (Q-Q plots and histograms) except for mortality parameters (logistic regression). In case of does that were either deceased or removed, data from the corresponding pen was excluded for analysis from that point onwards until the end of the remaining part of the reproduction cycle. As such, following treatments were excluded from analysis: two 4Y pens (cycle 1), three 4N pens (one in cycle 2, 3 and 4) and two 3Y pens (cycle 3).

Data collected between day 0 and 22 post-partum

Doe weight (between cycles) was analysed using a linear mixed model with group size, enrichment, their interaction and pregnancy (doe pregnant or not at the time of measurement) as fixed factors. In all the other statistical models, dependent reproductive performance variables measured before day 22 pp were assumed to have been potentially influenced by treatment during the previous group housing phase only. Kit number and weight on day 22 pp were analysed using a linear mixed model with group size, enrichment (both of the previous cycle) and their interaction as fixed factors. Pregnancy was added as an additional fixed factor. The same model was applied for the number of kits at birth (live and stillborn) but without pregnancy in the model. Kit mortality between birth and day 22 pp was analysed using a generalised linear mixed model (logit link) with group size, enrichment (both of the previous cycle) and their interaction as fixed factors. Pregnancy was added as an additional fixed factor. The same model was applied for doe using a generalised linear mixed model (logit link) with group size, enrichment (both of the previous cycle) and their interaction as fixed factors. Pregnancy was added as an additional fixed factor. The same model was applied for doe fertility (pregnant after AI) but without pregnancy in the model.

In all models, cycle, pen and doe ID were added as random factors. Interactions between variables were removed if non-significant (P>0.05). In the event of a significant group size, enrichment or pregnancy effect, a post hoc Tukey test was performed on the estimated least squares means to evaluate all pairwise differences.

Data collected between day 22 and 35 post-partum

Dependent reproductive performance variables, measured during the group housing phase (day 22 until 35 pp), were analysed with treatment (group size and enrichment) for the current reproduction cycle. Treatment of the previous reproduction cycle showed little effect on the results and was therefore excluded from the final models. Kit number and weight on day 35 pp and kit daily growth between day 22 and 35 pp were analysed with group size, enrichment, their interactions and pregnancy as fixed factors and cycle as random factor in a mixed linear model. Kit mortality between day 22 and 35 pp was analysed using a generalised linear mixed model (logit link) with the same fixed and random factors as the former model.

In all models, interactions between variables were removed if non-significant (P>0.05). In the event of a significant treatment or pregnancy effect, a post hoc Tukey test was performed on the estimated least squares means to evaluate all pairwise differences.

RESULTS

At the start of the fourth reproduction cycle, there was a shortage of four pregnant does to meet the requirement of 42 does with kits for the group housing phase on day 22 pp. Therefore, four does without kits were equally distributed so that only one pen per treatment had a doe without kits in group.

Data collected between day 0 and 22 post-partum

During the housing period in single-litter cages, 12 does were not retained for the experiment. Two does with an abscess and two does with mastitis were euthanised after veterinary medical evaluation. For five does, the cause of death remained unknown after visual inspection. Three does died after a fatal early abortion.

No significant effect of group size (P=0.48) or enrichment (P=0.62) were found for doe weight gain between reproduction cycles. Similarly, no effects were observed for doe fertility (pregnant after Al, %) (group size: $\chi^2=3.19$; P=0.07 and enrichment $\chi^2=0.48$; P=0.49), although a higher fertility percentage was observed in groups of four does as compared with groups of three does (Table 1). Significant treatment differences were absent for the number of liveborn kits (group size: P=0.20 and enrichment: P=0.74) and for the number of stillborn kits (group size: P=0.05 and enrichment: P=0.99). The number of kits at day 22 pp was not significantly influenced by group size (P=0.82) nor enrichment (P=0.99). Similarly, kit mortality (%) between birth and day 22 pp (group size: $\chi^2=0.06$; P=0.80 and enrichment: $\chi^2=0.29$; P=0.59) and kit weight at day 22 (group size: P=0.88 and enrichment: P=1.0) showed no significant treatment differences (Table 1).

Data collected between day 22 and 35 post-partum

During the experiment and while housed in group, seven does were removed from their pens. Four does (one 4N and 4Y and two 3Y) were severely injured and were thus removed from the group and treated for their lesions. Two does, one diagnosed with mastitis (4Y) and one with an abscess (3N), were euthanised after veterinary medical evaluation. One doe (4N) died of unknown causes. When housed in group, a total of 22 kits (1.6% of all participating kits) died of unknown causes. One and two kits were euthanised due to a fractured skull and an injured paw, respectively.

The number of kits at day 35 pp did not differ between treatments (group size: P=0.84 and enrichment: P=0.33; Table 2). Kit mortality between day 22 and 35 p also showed no significant difference due to group size (χ^2 =0.37; P=0.54) or enrichment (χ^2 =1.44; P=0.23). Similarly, kit weight at day 35 did not show a group size (P=0.22) or enrichment effect (P=0.53). Significant effects for daily kit growth were also absent (group size: P=0.07 and enrichment: P=0.33; Table 2).

Table 1: Overview of reproductive performance, measured between day 0 and 22 post-partum, for group size and escape enrichment. Sample size (*N*, single-litter cages) is indicated below treatment. Values represent least squares means±standard error.

	Group size (number of does)			Enrichment		
	3 (<i>N</i> =72)	4 (<i>N</i> =96)	P-value	N (N=84)	Y (N=84)	P-value
Doe weight gain (g)	148±31.3	124±28.8	0.48	127±29.8	144±30.3	0.62
Doe fertility (pregnant after Al) (%) ^a	80.8±7.5	92.1±4.4	0.07	89.5±5.5	85.2±6.4	0.49
Liveborn kits/litter ^a	12.4±0.36	11.9±0.33	0.20	12.2±0.35	12.1±0.34	0.74
Stillborn kits/litter ^a	0.08±0.15	0.49±0.14	0.05	0.29±0.15	0.28±0.14	0.99
Kits/litter, 22 d pp ^{a,b}	9.65±0.18	9.60±0.18	0.82	9.63±0.19	9.63±0.17	0.99
Kit mortality/litter, day 0-22 (%) ^{a,b}	5.01±1.46	4.72±1.36	0.80	4.52±1.37	5.32±1.44	0.59
Weight/kit (g), 22 d pp ^{a,b}	438±9.10	437±9.18	0.88	438±9.47	438±8.91	1.0

Switch from single-litter to group housing of three (3) or four (4) does with their kits 22 d post-partum (pp) until 35 d pp with (Y) or without (N) additional escape enrichment (PVC pipes and second level platforms).

^aTreatment of previous experimental reproduction cycle.

^bcalculated with standardised litter size after birth (9 kits/litter in the first reproduction cycle, 10 kits/litter in the second, third and fourth reproduction cycle).

Table 2: Overview of reproductive performance, measured between day 22 and 35 post-partum, for group size and escape enrichment. N=12 group pens per treatment within group size and enrichment. Values represent least squares means \pm standard error.

	Group size (number of does)			Enrichment		
	3	4	P-value	Ν	Y	P-value
Kits/litter, 35 d pp	9.07±0.32	9.01±0.32	0.84	8.89±0.32	9.18±0.32	0.33
Kit mortality/pen, day 22-35 (%)	1.07±0.50	1.42±0.57	0.54	1.64±0.64	0.92±0.45	0.23
Weight/kit (g), 35 d pp	1045±31.6	1026±31.6	0.22	1041±31.5	1031±31.7	0.53
Daily growth/kit, day 22-day 35	48.6±0.82	47.1±0.83	0.07	48.3±0.82	47.5±0.83	0.33

Switch from single-litter to group housing of three (3) or four (4) does with their kits 22 d post-partum (pp) until 35 d pp with (Y) or without (N) additional escape enrichment (PVC pipes and second level platforms).

Results calculated with standardised litter size after birth (9 kits/litter in the first reproduction cycle, 10 kits/litter in the second, third and fourth reproduction cycle).

DISCUSSION

In this trial, perhaps related to the limited number of repetitions per treatment, no significant effect of group size or escape enrichment were found on doe weight and fertility, litter size at birth, kit growth and kit mortality when does and their kits were housed part-time in groups. The reproductive performance of the does in our study are comparable with other reports on part-time group housing of rabbits. Doe fertility rates in the present study are comparable with Maertens and Buijs (2016) and Dal Bosco et al. (2019), who reported a fertility rate of 83.3% and 76.2% of part-time group housed does, respectively. Social stress related to group housing may affect the overall body condition and health of the does, especially when grouped in subsequent reproduction cycles (Dal Bosco et al., 2019). The first reproduction cycle in our experiment started with young and healthy does but by the fourth reproduction cycle not enough does with kits were available to meet the experimental requirements. This shortage can be explained by the loss or exclusion of does during the experiment for a variety of reasons: mastitis, unsuccessful Al, fatal early abortion, death by an unknown cause, or removal from the experiment due to injuries. After grouping, agonistic behaviour (including threatening, chasing, fighting and fleeing) among does usually takes place to establish a hierarchy (Rommers et al., 2011, 2013; Munari et al., 2020). The subsequent formation and breaking up of groups, and the associated stress and hierarchy fights, may take a toll on the does that are already subjected to an intensive breeding schedule. Furthermore, group housed does have been reported to consume less feed compared with singlelitter housing (Machado et al., 2019) and a lower ranking in the hierarchy may negatively affect the body condition of does compared to more dominant ones (Dal Bosco et al., 2019).

In the study of Maertens and Buijs (2016), 9.9 kits per litter at weaning were reported and a daily mean kit mortality of 0.14% was observed during the group housing phase when housing four does with their kits 18 d pp in group for a total period of 14 d. These results are similar to the present findings, i.e. daily average kit mortality (between day 22 and 35 pp) of 0.1% and 9.0 kits per litter at weaning. Furthermore, studies on group housing of four does from day 22 until 33 pp (Maertens and De Bie, 2017), day 2 until 33 pp (Zomeño *et al.*, 2018) and groups of six does from day 18 until 28 pp (Machado *et al.*, 2019) found similar litter sizes at weaning (8.2, 9.7 and 9.2 kits per litter, respectively). The large variation in experimental design, group size, timing and duration of the group housing phase may limit the accuracy of such inter-study comparisons.

In the wild and in high-density groups, kits are more often killed by does other than their mother (Rödel *et al.*, 2007). In the present trial, single-litter housing in the first part of the reproduction cycle protected the kits from early infanticide by unfamiliar does, but post-grouping kit mortality due to doe aggression could not be fully excluded. In comparative studies between single-litter and part-time group housing, kit mortality is usually higher in the latter (Maertens and De Bie, 2017; Dal Bosco *et al.*, 2019) but the proportion of mortality that can be attributed to doe aggression or other consequences of group housing (e.g. a reduction in nursing behaviour due to stress [Rommers *et al.*, 2012]) is not always unambiguous. During this trial, three kits were culled due to severe injuries acquired after grouping. In four cases, does had to be removed from the group due to numerous and/or severe injuries. The occurrence of culled or injured animals in part-time group housing systems has also been reported in other recent

studies. When multiparous does were housed in groups of four on either day 22, 25 or 28 until day 35 pp in the study by Van Damme *et al.* (2022), one doe and 0.5% of all kits were culled due to severe injuries. Huang *et al.* (2021) grouped four does without kits for an intended period of 15 d. The trial, however, was stopped after the tenth day in group due to ongoing aggression. The authors reported three culled does whose health was considered poor and two does which showed minor injuries. In contrast, in the study of Braconnier *et al.* (2020), multiparous does were housed in groups of six on either day 12, 18 or 22 until day 25 pp, but no injured animals required culling during the trial.

In this experiment, providing escape enrichment and altering group size (and stocking density) had no profound effect on reproductive performance of part-time group housed breeding does. Reduction in group size, even when major modifications were made, revealed no considerable improvements on the reproductive performance of breeding does. Future research could explore novel strategies with the goal of improving reproductive performance and animal welfare. A breeding strategy toward animals better suited for group housing systems and more insight is needed to reduce aggressive behaviour among does.

CONCLUSION

In this trial, providing escape enrichment (PVC pipes and elevated platforms) and altering group size (3 or 4 does) and concomitantly stocking density did not profoundly affect the reproductive performance of part-time group housed breeding does. Results from the present study were in accordance with other studies on part-time group housing in terms of doe fertility, the number and the weight of the kits. Doe aggression, however, remains a major drawback and needs to be reduced before part-time group housing can be implemented on a commercial scale. A better understanding of the social dynamics between does and novel breeding strategies may improve both reproductive performance and animal welfare.

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