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(Article begins on next page)

1 **Effect of different management protocols for grouping does on**
2 **aggression and dominance hierarchies**

3

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ABSTRACT

15

16 The study aimed at evaluating different management protocols of grouping does in regard to
17 aggressive interactions and the establishment of a hierarchy under commercial conditions. Fifty-seven
18 multiparous rabbit does of the Hycole hybrid maternal line were randomly distributed into three
19 different management protocols (MP) for a total of 5 consecutive trials: MP12 with grouping 12 days
20 after parturition, MP18 after 18 days and MP22 after 22 days. Video recordings were made during
21 the first 24 hours after grouping and after 6 and 10 days, and used to score aggressive interactions of
22 the animals with exception of treatment 3 where day 10 was omitted because it was after the weaning
23 of the kits. For MP12 and MP18 the frequency of different categories of aggressive interactions were
24 significantly higher on the day of grouping than afterwards, with a strong decrease on day 6 ($P < 0.02$),
25 but almost no change was found between days 6 and 10. This was especially true for biting, with a
26 high frequency of occurrence just after grouping and a subsequent decrease on day 6 under all

27 management schedules ($P=0.005$). The change in the number of aggressive interactions between 6
28 and 10 days after grouping was not significantly different between MPs. However, the time point of
29 6 days after grouping seemed to be fundamental in reducing the number of aggressive events.
30 Although a longer separation for 18 and 22 days after parturition did not reduce the total number of
31 aggressive interactions, MP18 and MP22 showed more mild aggressive interactions with respect to
32 MP12 on the day of regrouping. Moreover, when comparing MPs concerning the development of
33 hierarchy, hierarchies appeared very stable independently of the length of grouping ($P<0.01$).

34 **Keywords**

35 Rabbit does; Group housing; Management protocol; Aggression and dominance hierarchies;
36 Welfare

37

38 **1. Introduction**

39 Under natural conditions, the European rabbit (*Oryctolagus cuniculus*) is a social animal and
40 the typical group structure consists of an average of 2-9 females, 2-3 bucks and their litters (SurrIDGE
41 et al., 1999). Wild rabbits establish dominance ranks for access to breeding sites. Low-ranking does
42 are more stressed than high-ranking does as indicated by increased corticosterone challenge values,
43 as well as low reproductive success (Holst et al., 1999). Social behaviours of hybrid breeding does
44 kept for commercial purposes reflect natural behaviours, due to the limited domestication efforts
45 which have not resulted in marked behavioural differences from the wild rabbit (Trocino and Xiccato,
46 2006). In a Belgian park system where breeding does are group housed during part of the time,
47 physical contact between animals are infrequent and agonistic interactions occur especially after the
48 formation of the group (Buijs and Tuytens, 2015). However, in commercial farm conditions,
49 agonistic interactions between rabbit does commonly result in mild to severe injuries, reflecting
50 natural behaviour (Rommers et al., 2006). The reason is that in wild rabbits, separate dominance
51 hierarchies are maintained among males and females. The males' hierarchy is quite rigid, with the
52 dominant male, often larger and older than the others, having priority over females for mating and

53 the best places to feed and rest. On the contrary, the females' hierarchy is much less rigid and the
54 dominant females are more tolerant towards the other females of the group. However, during the
55 reproductive season, females can become extremely aggressive towards the same does with whom
56 they spent autumn and winter (Cowan and Bell, 1986). The most important resource for does is to
57 find a dry and safe place to build the nest, but these places may not be easily available within
58 commercial systems leading to fighting to establish the right to take over a particular site to give birth
59 (Mc Bride, 2004). Serious aggressive behaviours such as biting usually become less frequent once a
60 stable hierarchy has been established, and strong positive relationships develop between individuals,
61 who remain near each other and rest together (EFSA, 2005). Under commercial farming conditions,
62 when group housing is applied, females are usually regrouped with unfamiliar does, and this causes
63 the establishment of a new hierarchy through fights and aggressive interactions with consequently
64 high rates of injuries and low reproductive efficiency (Szendro and McNitt, 2012; Andrist et al.,
65 2013). On the other hand, conventional single housing of the domestic rabbits in small wire cages,
66 utilized until now in most European countries, often leads to stereotypic behaviours like hair-chewing
67 and biting bars, indicative of anxiety and frustration, as well as skeletal abnormalities (Lehman, 1991;
68 Gunn and Morton, 1995). To solve these welfare problems of rabbit breeding systems, members of
69 the European Parliament's Agriculture Committee voted in favour of a report that set out key
70 improvements for rabbit (growing and does) welfare in January 2017.

71 Regarding the Swiss farming system, group housing of breeding rabbit does is a requirement
72 for welfare friendly labels though results in many of the problems already introduced necessitating
73 alternatives. In this context, Andrist et al. (2013) found mild to severe lesions due to aggression in
74 up to one third of group-housed animals. The prevalence of injuries was especially high in the
75 management system with does individually separated after parturition for 12 days in order to prevent
76 fighting for nests, two litters in one nest box and pseudo pregnancy due to mounting does. Due to the
77 impossibility of performing basic ethological behaviours in single cages (Gunn and Morton, 1995)

78 and a high competition for the nesting sites before parturition and protection of litters from unfamiliar
79 does in colony housing (Mugnai et al., 2009), the industry lacks a management protocol that can
80 ensure a high level of animal welfare. As infanticides are restricted to the first 10 days after parturition
81 when females stay close to their burrows and are more aggressive towards other females in nature
82 (Rödel et al., 2008), does are individually housed during 12 days after parturition and group housed
83 thereafter. In contrast to other farm animals, such as pigs (Hoy et al., 2006) and cattle (Menke et al.,
84 2000), where the effects of regrouping animals have been extensively studied, there are few studies
85 on the development of hierarchy after the grouping of breeding does. Greater understanding of how
86 the dynamics of dominance evolves within the group over time would provide important information
87 to optimize group housing management systems.

88 The aim of this study was to find the best management protocol for regrouping (different
89 isolation periods to overcome the critical phase after parturition) does, to minimize social conflicts
90 and that can be applied on commercial farms. For this purpose, aggressive behaviour as well as the
91 establishment and stability of dominance hierarchies were observed..

92

93 **2. Animals, materials and methods**

94 *2.1. Animals and housing*

95 The experiment was carried out in a commercial rabbit farm in Geltwil (Switzerland), using a
96 total of 57 does of the Hycole hybrid maternal line that were not nulliparous, from August 2018 until
97 March 2019. Does were housed in groups of eight animals each, for five consecutive trials. They were
98 reared according to a Swiss animal-friendly label programme, which requires group housing of
99 females and a separated nest for each doe
100 (<http://www.blw.admin.ch/themen/00006/01715/01718/index.html?lang=de>). Each pen was
101 equipped with straw material and furnished with elevated platforms, hiding places, eight

102 compartments with nest boxes, drinkers and automatic feeders (Figure 1). Feed (UFA 925, UFA AG,
103 Herzogenbuchsee, Switzerland), water and hay were provided *ad libitum*.

104

105 2.2. *Experimental timing and management protocols*

106 For each of five trials, all animals were artificially inseminated (AI) on day 10 *postpartum*
107 (*pp*) and were housed individually from one day before parturition until day 11 *pp*. From this point,
108 does were divided into three different management protocols (MPs) (Figure 2), as follows: group
109 housing from day 12 *pp* (MP12), group housing from day 18 *pp* (MP18), group housing from day 22
110 *pp* (MP22).

111 To avoid the effect of parity order, in trial 1 all does were assigned to each MP semi-randomly
112 in a standardized way to ensure a similar distribution of parities. In consecutive trials, doe groups
113 were assigned to another MP. Does not pregnant as detected by manual palpation were replaced with
114 other animals. At least 2 does were replaced in each MP after each trial to achieve the group size of
115 8. No group remained stable between trials.

116

117 2.3. *Behavioural observations*

118 Following the methods of Andrist et al. (2012), video sequences were recorded and evaluated
119 (Figure 2) for each management protocol, as follows: MP12 at days 12, 18 and 22 *pp*, MP18 at days
120 18, 24 and 28 *pp*, MP22 at days 22 and 28 *pp*. All groups were observed during the first 24 hours and
121 after 6 and 10 days after regrouping, except for MP22 group in which day 10 was not present because
122 it was after weaning of the kits. All does were individually marked with livestock colour on their
123 backs and had numbered ear tags.

124 In accordance with Selzer et al. (2001), active behaviour is more common during dark hours
125 than during light hours, because rabbits are crepuscular animals. Therefore, two time-windows of 4
126 h each between 20:00 and 00:00 and between 04:00 and 08:00, respectively, were analysed for each
127 time point through video recordings with infra-red sensitive cameras. Based on an ethogram by Graf
128 et al. (2011), aggressive interactions were classified as biting (gripping with the teeth), boxing (hitting
129 with the front paws), chasing (aggressive following of another individual for at least three jumps),
130 ripping (two does kicking each other with the hind legs), carousel-fights (rapid chasing around and
131 around in one spot with the rear end of the opponent gripped between their teeth), threatening (quick
132 head movement towards another doe) and attacking (abruptly running towards a group mate).
133 Threatening and attacking were combined as mild aggressions because no body contact and hence no
134 injuries resulted. Likewise, biting and ripping were combined into a single response (severe
135 aggressions) as were chasing and carousel (without biting) (chasing behaviour).

136 For each agonistic event, the following parameters were recorded: type of aggressive
137 interaction, frequency of occurrence, the animal directing the behaviour (dominant subject), the
138 recipient of the behaviour (submissive animal), and location (classified as own nest, foreign nest,
139 platform, down) (Williamson et al., 2016). Frequencies of all interactions and durations of chasing,
140 ripping and carousel-fights were recorded. Aggressive interactions were considered to have ended
141 when each individual separated and engaged in different behaviours such as self-grooming, feeding
142 etc.

143

144 *2.3. Dominance hierarchy analysis*

145 The analysis of dominance within social animal structure has been a research focus since the
146 beginning of the last century (Schjelderup-Ebbe, 1922) and different methods have been used to
147 determine individual ranks from interactions. Among them, the Elo-rating method (Elo, 1978) tracks
148 rank measures as a consequence of wins and losses in encounters with other individuals: numerically

149 greater ratings indicate more successful competitors. Since Neumann et al. (2011) have published an
150 R function, use of Elo-ratings together with a stability index to model dominance hierarchies has
151 become one of the most useful methods in this field (Mc Donald and Shizuka, 2013).

152 For assessing the dominance hierarchy of each treatment along the five trials, the index of
153 stability (S) was calculated which ranges between 0 and 1, where 1 refers to stable hierarchies,
154 whereas values closer to 0 indicate more unstable hierarchies (McDonald and Shizuka, 2013).

155

156 *2.4. Ethical approval*

157 This study was approved by the Cantonal Office of Aargau (No. 30611) and met all cantonal
158 and federal regulations of Switzerland.

159

160 *2.5. Statistical analysis*

161 Statistical analysis was conducted using the software package SAS 9.4. Generalized linear
162 models on count data (Poisson distribution) with management protocol, trial, and day as fixed
163 categorical effects were computed using Proc Glimmix [SAS/STAT] Version 13.1 software¹.
164 Residuals were checked for normality. No videos existed for day 10 after regrouping in MP22 because
165 offspring were weaned before this day which made comparisons with the other protocols not possible.
166 Therefore, two sets of analyses were performed: 1) Comparing days 0, 6, and 10 after regrouping for
167 MP12 and 18 and 2) comparing days 0 and 6 after regrouping for all MPs. P-values were adjusted for
168 multiple comparisons by Tukey-Kramer. The relationship between the number of aggressive
169 interactions and time-of-day was analysed with the regression model including the linear and the
170 square term of time-of-day (Proc Reg). Only time points between 20:00 and 24:00 were included

¹ SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc. in the USA and other countries. ® indicates USA registration.

171 because only 3 aggressive events happened during the second time slot from 4:00 to 8:00. However,
172 the other analyses included all data.

173 Dominance hierarchy and its stability were calculated with R (version 3.6.0), using the
174 package EloRating (version 0.46.8, <https://cran.r-project.org/web/packages/EloRating/index.html>
175 [accessed 9-9-2019](#)). Results were assessed as significant when $P < 0.05$.

176

177 **3. Results**

178 Considering MP12 and MP18, the frequency of aggressive interactions decreased noticeably
179 from day 0 after regrouping, but no significant differences were detected between days 6 and 10
180 (Figure 3). Analysing the number of aggressive acts ($N = 30$), in the generalized linear model,
181 protocols did not differ (MP: $F_{1,4} = 1.07$, $P = 0.36$), whereas day and a day x protocol interaction
182 were significant (Day: $F_{2,8} = 13.78$, $P = 0.003$; Interaction: $F_{2,8} = 4.44$, $P = 0.05$). Regarding the
183 interaction, the variable day was significant only for MP18, but not for MP12 (MP12: $F_{2,8} = 1.28$, P
184 $= 0.33$; T18 $F_{2,8} = 25.01$, $P = 0.0004$). When day 10 was deleted in order to include all MPs, they did
185 not differ in the number of aggressive encounters (MP: $F_{2,6} = 1.96$, $P = 0.22$; Interaction: $F_{2,6} = 4.66$,
186 $P = 0.06$), however days differed (Day: $F_{1,6} = 31.08$, $P = 0.001$). The number of aggressive encounters
187 decreased from day 0 to day 6 ($F_{1,22} = 11.58$, $P = 0.003$, $N = 30$), but no effects of protocol ($F_{2,22} =$
188 0.24 , $P = 0.79$, $N = 30$) and trial ($F_{4,22} = 2.54$, $P = 0.06$, $N = 30$) were found when all MPs were
189 considered. Similarly, for MP12 and MP18, biting showed significant differences in respect of the
190 day of observation ($F_{2,22} = 6.92$, $P = 0.005$, $N = 30$), but protocol ($F_{1,22} = 0.2$, $P = 0.66$, $N = 30$) and
191 trial ($F_{4,22} = 1.47$, $P = 0.25$, $N = 30$) did not.

192 When all MPs were considered, MP18 and MP22 showed more aggressive interactions
193 classified as mild (threats and attacks), than MP12, on the day of regrouping (Table 1) (MP12 vs.

194 MP18: $t_8 = -3.73$, $P_{\text{adj.}} = 0.045$; MP12 vs. MP22: $t_8 = -4.16$, $P_{\text{adj.}} = 0.026$). On day 6, MPs did not differ
195 (all P-values above 0.39).

196 Only 3 out of 40 aggressive interactions occurred between 04:00 and 08:00. Disregarding this
197 time slot, the number of aggressive encounters increased with time from 20:00 to midnight ($F_{2,33} =$
198 7.11 , $P = 0.003$) with a linear ($t_1 = 2.4$, $P = 0.02$) and an exponential (square) term ($t_1 = 2.5$, $P = 0.02$)
199 (Figure 4).

200

201 *3.1. Dominance hierarchy*

202 The stability coefficients were very high (mostly above 0.6) and did not differ among MPs
203 ($F_{2,9} = 1.10$, $P = 0.38$). However, trials differed ($F_{4,9} = 6.38$, $P < 0.01$) (Figure 5). In trial 2, the Elo-
204 rating program did not generate a value of social stability for MP18 and MP22, possibly because the
205 number of interactions was too low.

206

207 **4. Discussion**

208 The aim of the present study was to investigate whether aggressive interactions of breeding
209 does can be reduced by keeping them isolated for more than 12 days after parturition. Being
210 gregarious animals, it would be desirable to keep rabbit does in groups for ethological reasons, but
211 this results in social conflicts with increase of stress and injuries, reducing their welfare and
212 performance (Rommers et al., 2006; Mugnai et al., 2009; Andrist et al., 2012). In addition, there can
213 be aggression towards offspring. As found by Mykytowycz and Dudzinski (1972), does tolerate their
214 own kits, but attack kits from other does. In this respect, Szendrő et al. (2012) recorded a high
215 frequency of bitten and injured litters by competitive does, reducing the chance of survival with lower
216 productive performances and less income for the farmer. However, Albonetti and Farabollini (1994)
217 found a large decrease in terms of aggressive interactions after the establishment of a hierarchy and

218 suggested that social interactions between rabbit does are mostly friendly over time, after a first period
219 of fights.

220 In our study, for MP12 and MP18, the frequency of different categories of aggressive
221 interactions were higher on the day of regrouping, with a dramatic decrease after 6 days. In contrast,
222 almost no change was found between 6 and 10 days suggesting that aggressive interactions ceased
223 after the social rank was established (Albonetti et al., 1990b; Andrist et al., 2013). It has generally
224 been observed that regrouping unfamiliar animals leads to an increase in aggressive behaviours at the
225 moment of group formation (Mykytowycz, 1958; Albonetti et al., 1990a) because a new dominance
226 hierarchy needs to be established (dominance aggression), and also probably to compete for resources
227 and space (territorial aggression) (Mykytowycz, 1958; Graf et al., 2011).

228 Only the factor number of days after parturition clearly affected the frequency of biting as the most
229 damaging aggressive interaction, with a high frequency of occurrence after regrouping and a
230 subsequent decrease in the course of the six-day observation period. Biting is considered a serious
231 interaction due to the potential for severe injury. Our findings confirm other studies (Mykytowycz,
232 1958; Lehmann, 1991) showing that, although aggressive chasing and submissive retreat remain
233 common, overt fighting becomes rare after the order of dominance has been established if the group
234 composition remains intact. This suggests that most of the agonistic encounters were caused by
235 dominance aggression because a new hierarchy needed to be established after the animals were
236 grouped, also caused by protection towards the litter (Szendro et al., 2012). In case of territorial
237 aggression agonistic encounters would remain frequent (Mykytowycz, 1958). Moreover, Larsen and
238 Grattan (2012) found that in mice prolactin induces neurogenesis in the female with critical changes
239 in the mood and behavior in the *postpartum* period. It is well known that prolactin has a complex role
240 in regulating aspects of maternal behaviour (Gonzà et al., 1996), therefore can be probably involved
241 in aggressions, aimed at protecting the kits. When correlating hormonal regulation of maternal
242 behaviour with lactation curve of rabbit does, there seems to be a link between the timing of

243 aggressions and milk production, as previously reported by Zomeño et al. (2018). In fact, in our study,
244 MP12 showed the highest frequency in severe aggressive interactions, probably linked to the highest
245 milk output, respect MP18 and MP22 that showed more mild aggressive interactions, probably due
246 to the descent phase of the lactation curve. In rabbits the curve of lactation is asymmetric with a
247 convex ascending and a concave descending period (Lebas, 1968) after the peak of lactation on day
248 18-19 after parturition (Lebas, 1968).

249 However, when considering all types of aggression, fewer severe aggressive interactions were
250 present in MP18 than in MP12. In particular, MP18 and MP22 showed more mild aggression without
251 body contact than MP12 on the day of regrouping. Therefore, the level of aggression seemed to be
252 affected by the durations of separation between does, following parturition, probably due to the
253 greater age of kits that were, after a longer isolation, less vulnerable and so does don't need to apply
254 severe aggressive interactions like biting or boxing to protect them.

255 The change in the frequency of biting between 6 and 10 days after regrouping was not
256 significantly different between protocols, suggesting that a hierarchy was established within a few
257 days and biting mostly stopped.

258 Aggressive interactions were most frequent in the dark hours following regrouping, in fact
259 hardly any aggressive interactions were recorded during light hours (8.00-20.00) (unpublished data).
260 Since the trials were spread from late summer to spring the relationship between time-of-day and
261 amount of aggressive interactions is difficult to interpret, but the highest frequency was found close
262 to midnight when it was always dark. This can be a problem for farm management because the farmer
263 might not be aware of the aggressive behaviours.

264

265 *4.1. Dominance hierarchy*

274 When animals form social groups, it is possible to determine their order within the dominance
275 hierarchy (Hinde, 1976). According to Elo (1978), individuals with similar Elo-ratings (and thus
276 competitive abilities) may be considered to belong to the same category or class, while dissimilar
277 Elo-ratings are predictive of clear dyadic dominance relationships. To the best of the authors'
278 knowledge, there have not been any studies on the strength or stability of hierarchies in breeding
279 rabbit does before. The observed stabilities were higher than 0.6 in most trials, which indicates stable
280 hierarchies (McDonald & Shizuka (2013). Moreover, our data displayed a strong effect of trial period
281 which might be due to season since the first trial was carried out in September, the third and fourth
282 in the winter and the fifth in February/March. It appears that the groups outside the natural breeding
283 season, namely in the winter, had lower stabilities. Stability might result from higher aggressive
284 interactions due to a higher level of testosterone, confirmed by Birganti et al. (2003) where an
285 increased testosterone induced agonistic interactions in dominant rabbits. Does had smaller
286 anogenital distances in the winter trials which supports this interpretation (Michèle Braconnier,
287 personal communication).

288

289 **5. Conclusions**

290 In Switzerland rabbit does are usually separated for 12 days after parturition to avoid
291 infanticide and pseudopregnancy. Our results indicate that a longer separation for 18 and 22 days did
292 not reduce the number of total aggressive interactions, but a longer separation than 12 days was
293 important to reduce severe behaviours between does. Additionally, the time point of 6 days after
294 regrouping resulted to be crucial for the reduction of total aggressive interactions, as time frame
295 necessary to establish the hierarchy. Maybe it would be better to give access to the other does
296 gradually e.g. grouping them during the daylight with separation during the night, since hardly any
297 fights occurred during the light hours (unpublished data) being crepuscular animals and so more
298 active at night, thus having a gradual approach to regrouping the animals.

299 Moreover, to develop a suitable protocol for does regrouping after parturition, and also to give
300 important suggestion for housing systems in terms of welfare, further investigations (physiological
301 evaluations) should be performed to complement the hierarchy stability measurements, analyzing the
302 possible correlations between milk production (lactation curve), associated hormonal changes and
303 maternal behaviour (kits protection).

304

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309

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