World Rabbit Science

World Rabbit Sci. 2016, 24: 321-326
doi:10.4995/wrs.2016.3978

DEVELOPMENT AND ESTABLISHMENT OF AN INDIVIDUAL AGGRESSIVENESS TEST PROTOCOL IN BREEDING DOES

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Abstract: The present work aims to define the optimal conditions to perform a resident-intruder test in individually housed breeding does as a measure of aggressiveness and describe the biological characteristics of aggressiveness in rabbit does: severity, frequency, duration and latency of aggressive events. Sixty-four nulliparous does at 90 d of age were used for this experiment, half (32 does) as residents and the rest as intruders, testing them once a week for 14 wk. The time and duration of each aggressive behaviour was recorded and analysed to assess the aforementioned measures. According to the results, and regarding the studied effects, the origin of the animals caused no effect, whereas the level of aggressiveness seemed to be clearly increased in weeks 3-7 of the experiment, when animals were 110-140 d of age. In conclusion, a resident-intruder test lasting 1 min is enough to assess individual aggressiveness in adult breeding does, the response of which evolves with age and repetition.

Key Words: aggressiveness, behaviour, rabbits, resident-intruder.

INTRODUCTION

Adult rabbits are strongly territorial, at least when they are living in groups (Dudzinski *et al.,* 1977). However, Heath (1972) suggested that individual aggressive events, which may be predominantly territorial for the wild rabbit, become confused with events of sexual behaviour in the laboratory rabbit. This can be related with the fact that aggression in rabbits is usually intrasexual, although females are less tolerant with unfamiliar conspecifics and with young rabbits (Farabollini *et al.,* 1991).

Nevertheless, these aggressions, often preceded by investigatory behaviour (approaching and sniffing) (Albonetti *et al.,* 1991), can also be related to the establishment of social structures, and the presence of a novel conspecific in an established group induces behaviours usually performed under unstable social conditions (Farabollini *et al.,* 1991). Thus, when a rabbit is introduced into a pen permanently occupied by another individual, a contest for dominance develops (Dudzinski *et al.,* 1977). The intensity of reactions to intruders appears influenced by the social rank of resident females and the sex of the intruder, but in general it is considered that high-ranking females actively defend their territory or social status (Farabollini *et al.,* 1991).

All these findings referred to group housing systems, but in accordance with the supposed territoriality of rabbits and the tendency to establish hierarchies in wild conditions, it is possible that individually housed rabbits might behave in a similar way when challenged.

Despite this, nowadays there is a tendency to promote group housing of breeding does as an important factor of well-being (Princz *et al.,* 2008), although several problems such as aggression might appear (Rommers *et al.,* 2006). The incidence of aggressive behaviour increases with age (Princz *et al.,* 2008) and it will occur when mixing the does for the first time (Rommers *et al.,* 2006). Consequently, the age at which this mixing is performed might be important and needs to be studied accurately.

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doi:10.4995/wrs.2016.3978

Individual recognition between animals forms the basis of the territorial behaviour identifying individuals or groups. In addition, olfaction is the dominant sense in most mammals (Brennan and Kendrick, 2006). These aspects might suggest the idea that rabbits can behave differently depending on their previous experiences. This could mean that aggressiveness towards a rabbit located in the same room might be different to that directed against a rabbit housed in a different room.

The resident-intruder test is widely used to assess aggressiveness in animals, mainly in pigs and rodents (e.g. Erhard and Mendl, 1997; D'Eath and Lawrence, 2007; Trent *et al.,* 2009) and Farabollini *et al.* (1991) found it useful to assess aggressiveness in groups of female rabbits). This test of individual aggressiveness measures the individual's propensity to start an offensive encounter (Erhard and Mendl, 1997). Nevertheless, there is a lack of information on development of the protocols in the literature.

Thus, this work has 2 main objectives: first, to define the optimal conditions to perform a resident-intruder test in individually housed breeding does as a measure of aggressiveness, and second, to describe the biological characteristics of aggressiveness in rabbit does: severity, frequency, duration and latency of aggressive events.

MATERIAL AND METHODS

Animals

Sixty-four nulliparous does (90 d of age) from synthetic line V from the Universidad Politècnica de València (Baselga, 2002) were used for this experiment. The animals had just started their sexual development and behaviours, which occurs at around 70 to 84 d according to Rommers *et al.* (2006), or 60 to 70 according to Lebas *et al.* (1996), before the onset of puberty. The study was carried out in an experimental house located in Segorbe (Castellon, Spain), equipped with mechanical ventilation. The does were individually housed in cages with the following dimensions: 40×100×35 cm (width×length×height).

Resident-intruder test

Half of the subjects (32 does) were tested as residents and the rest were tested as intruders. To avoid familiarity between animals, does were never confronted twice with the same opponent, with a litter mate, or with an animal housed in an adjacent cage. Intruders came from 2 different sources: 16 intruders came from cages inside the same experimental room as the residents, and 16 came from an adjacent room. All tests were carried out inside the residents' home cages (100×40×35 cm cages).

Each doe was tested once a week over 14 wk (from 90 to 190 d of age) in a different order each time. Not all does were tested on the same day of the week, so tests could always take place during the same period of the day. The intruder was taken from its cage, marked and introduced in the resident's cage. The behaviour of the animals was recorded for thirty minutes by placing a video camera in front of the cage, so that animals could not see humans during the test. Afterwards, video records were observed continuously, and behavioural observations began upon introduction of the intruder. The categories shown in Table 1 were considered (taken from Farabollini *et al.,* 1991).

The time at which each behaviour started and its duration were recorded in order to assess the latency and duration of each interaction. All tests were performed in the morning, when rabbits are likely to be resting and less active according to their endogenous nocturnal habits (Jilge, 1991).

Table 1: Description of aggressive behaviours considered in the resident-intruder tests.

Behaviour	Description
Threatening	abruptly orientating head toward a conspecific with half-closed eyes and mouth ajar
Attacking	abruptly running towards a conspecific, invariably followed by other aggressive items
Chasing	running towards a conspecific that runs away
Biting	gripping or tearing a conspecific with the teeth
Confrontation	reciprocal wrestling with biting and repeated kicking with fore and hind leg
Chin-marking	rubbing chin on a conspecific (according to Heath, 1972, who considered it a territorial function)

The test was interrupted and the rabbits separated when either doe attacked the other for more than 10 s. If no attack occurred, the test ended after 30 min.

Statistical analysis

Statistical analyses were performed using SAS (2009) and the level of significance was set to 0.05.

To perform the analyses, 2 variables were constructed from the different behaviours registered during the test.

- Fight (F): including all aggressive behaviours in Table 1 lasting less than 10 s.
- Serious Fight (SF): F that lasted more than 10 s, and consequently meant the interruption of the test.

To characterise the test, a descriptive analysis of the data through a frequency analysis (Proc FREQ) was carried out. Percentage of encounters interrupted due to SF, percentage of residents which showed SF during the test, percentage of encounters which showed F and number of F during the tests were calculated. Means and standard errors (SE) were calculated for latency to the first attack, latency to the first F, latency to SF, and duration of F (Proc MEANS). Means and standard errors were calculated for frequency of F and SF (Proc ANOVA).

Occurrences of F and SF were analysed using a repeated measures logistic regression (Proc Genmod) with age (or evolution of the response, as the effect is confounded with test repetition) and intruder's origin as explicative variables.

The effect of the intruder's origin and evolution of the response on latency to the first attack (any of the aggressive behaviour described in Table 1) was analysed through a generalised linear model (Proc GLM). Latency did not behave normally and logarithmic transformation was performed. Latency to SF was assessed using a Poisson regression (Proc GLIMMIX).

Stability of latency to F with the evolution of the response (age) of the rabbits and the effect of the precedence was also analysed with a repeated measures general linear model (Proc MIXED), with age as the within subject factor and the resident animal as the experimental unit.

RESULTS

Test characterisation

An SF was only observed in 52.62% of the encounters. However, all residents had to be separated at least once due to an SF during the span of the experiment. When encounters with at least one F were studied, the percentage of incidence increased up to 72.31%.

The number of F recorded during the encounters is displayed in Figure 1. As can be observed, encounters with 1 and 2 F were the most frequent, even though there were encounters with up to 11 F. Tests which had to be stopped due to an SF were not included in this analysis.

The results of the latencies and duration of the fights are reported in Table 2. As shown, the latency to the first SF is considerably higher than to the first attack, and more time is needed for the occurrence of a fight (both F and SF). The SE in the latency to SF is high because it includes both the animals that react instantaneously to the intruder, and those that do not react at all. In addition, the mean duration of the F is 3.15 s.

These results might be helpful in further studies to establish a protocol for the aggressiveness test.

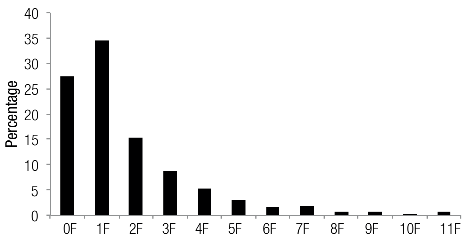

Figure 1: Frequency for the number of fights observed in the encounters during resident-intruder tests. Stopped fights were not included in these data.

Table 2: Latencies to each type of attack and duration of the fights during the resident-intruder tests.

Variable	Mean±SE (s)
Latency to first attack	24.68±1.12
Latency to first F	280.41±12.85
Latency to SF	295.01±430.60
Duration of F	3.15±2.10

SE: standard error. F: fight. SF: serious fight.

Effect of evolution of the response (age) and origin of the animals on aggressiveness

As shown, test week only affected the probability of an F occurring (β=0.0247, *P*>0.0001), but not an SF (β=–0.0048, *P*=0.1354). However, the origin of the intruder varied this probability both in F and SF. According to the intercepts, the fact that the intruder belonged to the same experimental room increased the probability of an F (β=0.7315, *P*=0.0051) whereas it decreased the probability of an SF (β=–0.4283, *P*=0.0027).

Latency to the first attack is shown in Figure 2. In this case, evolution of response resulted statistically significant (*P*<0.0001) whereas the origin of the intruder did not (*P*=0.1664). As can be drawn from the figure, latency suffers a strong decrease until 110 d of life of the breeding doe and then, until 140 it is inconstant. From day 140, it reaches a low level which is maintained from this moment on.

Figure 3 shows the results concerning the latency to an SF. It can be observed that it does not have a clear decreasing pattern, although from day 148 it seems to be lower than at the beginning of the tests (*P*<0.0001). In fact, it can be observed again that by day 110 there appears to be a decrease in the latency compared to the previous days, although in the following days this pattern is not consistent. In addition, the origin of the intruder did not have a significant effect on this latency either (*P*=0.1261).

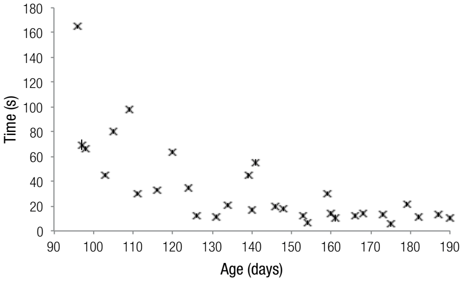

Figure 2: Evolution of latency to first attack expressed as mean±standard deviation during the days in which the resident-intruder test was performed.

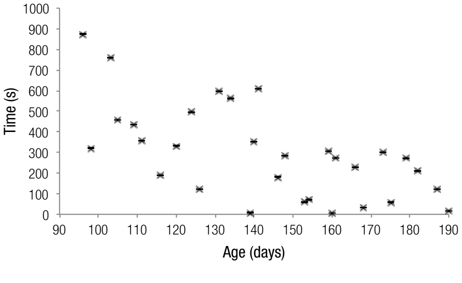

Figure 3: Evolution of latency to a serious fight expressed as mean±standard deviation during the days in which the resident-intruder test was performed.

The evolution of the frequency of F per encounter and week of the experiment is reported in Figure 4. As can be observed, there is a significant increase in the frequency of F, reaching a maximum in week 13, when does are 181-188 d of age, and an apparent plateau from week 7 (139-146 d of age) onwards (*P*<0.0001). The evolution of the frequency of SF per encounter was not statistically significant, so it was not reported (*P*=0.8909).

DISCUSSION

According to the results obtained, as the first attack took place within 1 min approximately and latencies to F and SF were 4.7 and 4.9 min respectively, a resident-intruder test conducted to assess the individual aggressiveness of an adult rabbit in the way explained in the present work can be performed in a span of 5 min. Nonetheless, given the high SE of the latency to SF (7.2 min) it is recommendable to extend the test duration up to 10 min. This proposed time is the same as some authors used for other species (Alves *et al.,* 2014; Yang *et al.,* 2015), although some others propose lower times (Erhard and Mendl, 1997, 3.5 min; D'Eath and Pickup, 2002; D'Eath, 2004; Rodríguez-Arias *et al.,* 2014; 5 min), which highlights the lack of establishment of a protocol. However, we must emphasise that this time can be different when housing conditions change, as reported by Farabollini *et al.,* (1991), who proposed a time in group housing of 15 min, as the dominance relations between the animals in a group influence the test.

In addition, the time during which the SF is considered can be reduced in accordance with the results obtained. There were 2 types of fights: those which lead to the end of the test (SF) and those which did not lead to it (F), whose mean duration was 3.15±2.1 s. Thus, it can be assumed that most F longer than 5 s finish in an SF, and the test can be ended if the F lasts longer than this instead of after 10 s as initially proposed, avoiding unnecessary animal suffering.

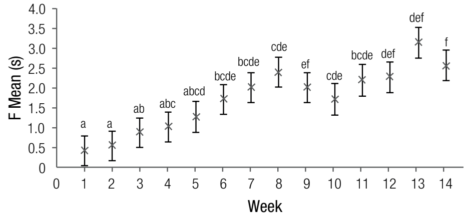

Figure 4: Mean frequency in seconds (±standard error) of fight per encounter and week. [abcdef] Means not sharing letter were significantly different at *P*<0.05.

Results indicate that the aggressive response clearly evolves week after week, and in particular, the older the rabbit (or the more times the test is repeated, as this effect is confounded) the more aggressive it is, as studied latencies decrease by over 50%, and F frequency increases more than fourfold. Dudzinski *et al.* (1977) established the maximum level of aggression between 61 and 90 d of age, which more or less coincides with the onset of sexual development and behaviours, which occurs around 70 to 84 d, according to Rommers *et al.* (2006) or 60 to 70 according to Lebas *et al.* (1996). However, our work established that it is from 110 d (some days before the onset of puberty, according to Lebas *et al.,* 1996) and 140 d (puberty totally established) when the level of aggressiveness is increased, as latencies to different fights decrease substantially, and frequency of F increases substantially as well. This would be in line with that previously reported by Hoy and Schuh (2004). From these days on, the decrease in latency is attenuated and the level of aggressiveness maintained, but the reasons why this aggression may persist are still unknown (Chu *et al.,* 2004).

However, results regarding the origin of the animals suggest that it has no clear effect on the aggressiveness of the animals, as results are confusing: the logistic regression showed differences between origins, but these findings are not consistent with the rest of the results, as it has been reported that non-statistically significant differences were found for latency to first attack or to an SF. It seems then that the fact that the breeding does share a location does not contribute to reduction of the aggressiveness level as a consequence of the possible familiarity. This might be because the recognition between individuals only comes about as a consequence of the secretion of the inguinal glands, and the "group odour" obtained consequently (Andrist *et al.,* 2014) is only effective at a short distance. Bearing this in mind, and as aggressions between conspecifics is one of the main problems in group housing of breeding does (Szendro and McNitt, 2012), the results obtained in this study can be an approach to the mixing of animals.

CONCLUSIONS

In conclusion, this work allows the definition of the optimal protocol of a resident-intruder test for breeding does, and also suggests that the aggressive response of these animals clearly evolves, although whether it is caused by age or by repetition effect is not clear, as the two are confounded, the level of aggressions increasing between 110 and 140 d of age (or weeks 3-7 of the test). On the other hand, as the effect of the intruder's origin is not significant, this parameter does not need to be taken into account when performing the test.

Aggressiveness in breeding does is an individual trait and occurs when the animals are subjected to a resident-intruder test. This test can be performed with a total duration of ten minutes, as most of the aggressive behaviours are observed in this timespan. Thus, it is possible to characterise the aggressiveness in rabbit does through this test using three items: first fight, fight, and serious fight.

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