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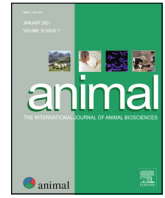
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Effect of access to outdoor grazing and stocking density on space and pasture use, behaviour, reactivity, and growth traits of weaned rabbits



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ABSTRACT

In a context of evolving concern over housing conditions of farmed rabbits, we developed a housing system that allows access to an outdoor area. The aim was to study the health status, growth and behaviour of rabbits raised at two stocking densities with access to a paddock, or not. We distributed 299 weaned rabbits in four groups (**YH**: 100, **NH**: 99, **YL**: 50 and **NL**: 50) using a 2×2 factorial design including access (**Y**: yes) or not (**N**: not) to a 23 m² paddock and the indoor stocking density (**H**, high: 17 or **L**, low: 9 rabbits/m²). We measured the growth and health status of each animal weekly for 42 days (from 31 to 73 days of age) and performed reactivity tests to a new environment, a human and new object. We also assessed the rabbits' behaviour at days 26 and 40 by doing a visual scan of each animal at regular time intervals. Our results showed that stocking density had no effect on mortality, but mortality tended to increase with outdoor access from 3.0% to 7.0% ($P < 0.10$). Although the stocking density had no effect on average daily gain, it was higher in rabbits in the N group than in the Y group (+3.6 g/day; $P < 0.05$). Rabbits entered the paddocks for the first time in less time at the beginning of the trial (50 s at day 3 vs 10 min at day 31; $P < 0.001$). The proportion of rabbits outside after 20 min of the new environment test was higher among rabbits in the L group than in the H group (+24% points at day 3 and +11% points at day 20; $P < 0.001$). Regardless of the stocking density, more rabbits in the N group touched the experimenter's hand (16% vs 27%; $P < 0.05$) and the new object (34% vs 20%; $P < 0.05$) than rabbits in the Y group. Inactivity was more frequent in rabbits inside the pens than in the paddocks (70.0% vs 34.2% at days 26 and 40; $P < 0.05$). Locomotion was more frequent in the paddocks than in the indoor pens (20.0% vs 7.2% at days 26 and 40; $P < 0.05$). The stocking density did not affect the behavioural traits measured. In conclusion, providing rabbits access to a paddock could allow them to fulfil some natural behaviours but slightly reduced their growth.

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Implications

Intensive livestock farming systems are criticised by citizens regarding animal welfare, mainly for rabbit reared in cages. The absence of outdoor access is a particular concern. We propose an alternative system that enables access to a paddock. It is intended to be profitable and easily manageable for farmers, while diversifying the behavioural repertoire compared to indoor production. We provide reference values for growth traits and mortality, the rabbit's reaction time to novel stimuli and their behaviour at two stocking densities. Under this system, growth and mortality are acceptable and rabbits express most of their specific behaviours.

Introduction

The living conditions of farm animals are growing societal concerns in Europeans (Delanoue et al., 2018), who want to be sure they are purchasing products from animals kept in conditions that respect animal welfare. More specifically, Europeans denounce the use of cages, the lack of free access to outdoors and the high stocking density practised in industrial farming systems. In this societal context, some consumers choose to eat less meat, while others only consume ethically distinctive meat from free-range animals (de Boer et al., 2008). More committed citizens plead for the end of the "cage era" (Eck, 2017).

Rabbit farming is not an exception as the vast majority of individuals spend their entire life in wired cages with no, or limited enrichments and are raised at high-density rate. In France, for example, the legislation allows rabbit farmers to house up to 24 rabbits (with a live weight of 1.9 kg) per square metre (or 45 kg

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per m² at 60 days of age; AFNOR NF V47.001). The European Food Safety Authority (EFSA Journal, 2020) concluded that caged rabbits of all ages lack comfort and have limited space to express their natural behaviours, including locomotion.

Alternative housing is available for industrialised rabbit production systems, examples being structurally enriched cages (so-called “welfare cages”), elevated pens and floor pens. However, complete or partial access to outdoors is only used in niche systems, including “label rouge” and organic rabbit production (EFSA Journal, 2020). In these niche systems, the variety of housing solutions makes it difficult to provide a standard reference. Although alternative rabbit production systems are considered to better respect rabbit welfare, scientific knowledge of both behavioural and productive traits is limited. By offering more space, access to outdoors allows the rabbit to express a wider range of specific behaviours (like running, hopping, jumping), gives them access to natural light (removed in windowless building), and reduces the level of fear compared to rabbits raised indoor (d’Agata et al., 2009). However, it also reduces growth (Pinheiro et al., 2012; Loponte et al., 2018) and may impair the health status of animals that come into direct contact with their excrement (Lambertini et al., 2001). It is therefore a scientific challenge to understand the combination of elements that would better balance welfare, health, and productive traits of rabbits in systems that allow outdoor access.

Several indicators are required to correctly assess the welfare of rabbits with access to an outdoor system. In addition to noting the spatial distribution and the frequency of specific behaviours, a reaction time test (the time needed to explore a new environment and to approach a human or a novel object) provides valuable information on the emotional state of rabbits. It helps characterise emotions (like fear) when the rabbits are exposed to a new and unknown stimulus (Verga et al., 2007). Fear can result in immobility, attempts to escape or alertness to humans (EFSA Journal, 2017). A longer reaction time to interact with a human, to touch a novel object, or to explore a new environment is considered as an indicator of fear and anxiety. Together with health and productive traits, these measures help construct a holistic view of rabbit housing systems.

Given the increasing concern about how rabbits for meat production are housed, we developed a housing system that aims to combine the benefits of the indoor floor pens for productivity and health with the benefits of access to an outdoor grazing area for animal welfare. The study focused on the effect of access to an outdoor grazing area and stocking density on the health status, the growth and the behaviour of weaned rabbits. We hypothesised that rabbits having an outdoor access will be more active and will have a more diversified behavioural repertoire with no detrimental effects on their growth and health.

Material and methods

Animals and experimental design

A total of 299 rabbits (INRA strain 1777) of both sexes were reared for 42 days starting from weaning (experimental days 0 to 42; rabbits were thus aged 31–73 days) in a 30 m² mobile poultry shed (SAS DI.ST.EL, 81340 Valence d’Albigeois, France; W × L × H: 500 × 600 × 250 cm). The mobile shed was placed in a pasture located in Pompertuzat (south of France) in the landing routes of the Toulouse-Bagnac Airport (30 km away). It was equipped with eight roofless pens (PARCLAP26; Chabeauti, 79330 Glénay, France; W × L: 100 × 200 cm each with two 45 × 100 cm platforms; Fig. 1). The longitudinal walls of the mobile shed were pierced with four hatches allowing access to the paddocks and placed in front of each



Fig. 1. (A) The mobile poultry shed located in a pasture. The four brown squares in the bottom part of the shed are the exit hatches that allow the rabbits access to the paddocks (two hatches were closed). (B) Two pens (2 m²) inside the portable shed.

pen. Sunrise was around 7000 h and sunset was around 2000 h. No artificial lighting was applied in the building.

At weaning, rabbits of both sexes (pink strip on the male’s forehead) were randomly distributed in four groups (YH: 100, NH: 99, YL: 50 and NL: 50) according to a 2 × 2 factorial design, including access (Y: yes) or not (N: not) to a 23 m² paddock (W × L: 2.9 × 8 m) and different indoor stocking densities (H, high: 17 or L, low: 9 rabbits/m²). 50 or 25 rabbits were kept in each pen (W × L × H: 100 × 200 × 80 cm). Rabbits of the Y groups were given continuous access to a paddock from day 6 (37 days of age) except during the reactivity tests (see below).

The outdoor area was protected from predators by wire mesh (25 × 25 mm), a three-wire electric fence and an aviary net. The pasture itself was delimited by a wire fence (100 × 100 mm mesh). Y groups had access to a meadow sown in autumn 2018 with a forage mix (OH-43RM, Otto Hauenstein Seed SA, 13H Orbe, Switzer-

land). Throughout the experiment, the rabbits also received a commercial diet for growing rabbits (STABI-GREEN G, Terrya, Rignac, France) containing 11.3 MJ of digestible energy per kg of DM, 17.8% of CP, 2.8% of fat, 40.1% of NDF, 22.7% of ADF and 7.9% of ADL on a DM basis. This diet contained 66 mg/kg of robenidine hydrochloride to prevent coccidiosis. To reduce the risk of digestive disorders, rabbits followed a feed restriction programme: 85 g/kg of live weight (measured at day 0) per day during the first week then an additional 15 g/rabbit every 7 days until experimental day 42. The amount distributed was adapted to the real number of rabbits in each pen, i.e. it took into account the mortality in each pen. In each pen, the feeder was 87 cm long, 26 cm high and 13 cm wide. Rabbits were weighed individually once a week between experimental days 0 and 42 and their health status was checked at the same time. Mortality was monitored daily.

Ambient parameters and grassland areas

The temperature inside and outside the shed was recorded daily. Rainfall and wind speed were also monitored daily from the INRAE CLIMATIK platform (<https://intranet.inrae.fr/climatik/>) managed by the AgroClim laboratory (Avignon, France). Herbage height (measured with a grazing stick at 25 points along two transects per paddock) and botanical composition (proportion of legumes or grass in eight 0.25 m² plots) were measured in each paddock (two for the YH and two for YL group). These measurements were taken 1 day before the animals arrived, and 21 and 35 days after their arrival (corresponding to the rabbits' age of 30, 58 and 72 days, respectively). To estimate plant biomass in the paddocks, four 25 cm² areas were taken at a distance of 0.5 m, 2 m, 4 m and 6 m from the exit hatch 1 day before the arrival and 1 day after the departure of animals (corresponding to the rabbits' age of 30 and 74 days, respectively). Inside the plots, the grass was entirely cut at a height of 2 cm from the ground. Samples were pooled and placed in micro-perforated bags and stored at 4 °C. The samples were subsequently analysed using the methods described by the EGRAN (2001) group as the best suited for rabbit nutritional experiments: DM (24 h at 103 °C; ISO 6496.1999 method), ash, crude protein (N × 6.5, Dumas method, ISO 16634.2004 method) and fibres (ADL, ADF and lignin, Van Soest et al., 1991 method) were determined.

Spatial distribution

Spatial distribution of rabbits in the pens and in the pasture was evaluated six times a week (3 days a week and twice a day) for six weeks: on Mondays before feeding 8000 h and at 1400 h, on Wednesdays at 1000 h and at 1500 h and on Fridays at 1100 h and at 1600 h. Rabbits were counted in three distinct areas in the pens: on the platforms (corridor and hatch platforms), on the ground floor between the two platforms (floor between the platforms) or under the platforms (corridor and hatch) and in two distinct paddock areas located at different distances from the exit hatch: (A1) from 0 to 2 m or (A2) from 2 to 8 m. Before observation of each spatial distribution, ambient temperatures near hatches and hall were checked with an infra-red-light device (Fluke®, TiS45, Everett, WA USA).

Reactivity and behavioural evaluation

New environment test

The test was used to investigate the reactivity of animals to a new environment (outdoor paddocks) at experimental days 3, 20 and 31 (rabbits aged 34, 51 and 62 days, respectively) for 20 min before feeding according to the method of Bertrand (2002). Animals in the Y group were tested at day 3 and animals in the N

group at days 20 and 31 (one pen per group (H and L) on each of these 2 days). The latency time for the first rabbit to go outside (i.e., the rabbit's whole body was outside the pen) was measured and the number of rabbits in the pastures (counted at 5 min intervals) is expressed as the percentage of rabbits in the pen and per sex.

Human approach test

The test was performed on days 5 and 32 of the experiment (rabbits aged 36 and 63 days, respectively) in each pen according to the method of Verwer et al. (2009). The aim was to compare the reaction of the rabbits to a human depending on whether they had outdoor access or not (Y or N). At day 32, rabbits were all gathered in their pens and hatches were closed during the test. After lowering the side door of the open, a trained operator with whom the rabbits were familiar placed his hand in the centre of the pen approximately at a height of 10 cm above the floor (i.e., at the height of the animals' withers). Latency time to first contact with the operator was measured and the number of rabbits that touched or approached his hand (to a distance of less than 10 cm) for 5 min is expressed as a percentage of rabbits in the pen and per sex.

Novel object test

This test was used to compare the reactivity to a new stimulus between rabbits with outdoor access or not (Y or N) according to the method of Verwer et al. (2009). The test was performed at days 4 and 33 of the experiment (i.e., in rabbits aged 35 and 64 days, respectively) for 10 min while the exit hatches were closed (only for the test period). An operator gently clapped his hands in the paddock to make the rabbits enter the building while another closed the hatches. A full red painted plastic bottle (H × Ø: 32 × 10 cm) anchored with a string to the cap was suspended from the roof into the centre of each pen at a height of approximately 10 cm above the floor. The latency time to first contact with the object was measured and the total number of rabbits that touched or approached the object (to a distance of less than 10 cm) is expressed as a percentage of rabbits in the pen and per sex.

Behavioural evaluation

The behaviour of animals was assessed for 10 min using the scan sampling method with video recordings of all groups inside the mobile shed combined with direct observation of the YH and YL groups in the paddocks at days 26 and 40 of the experiment (rabbits aged 57 and 71 days). Observations were made in the morning (between 0700 and 0800 h just after sunrise), in the afternoon (between 1400 and 1500 h) and in the evening (between 1900 and 2000 h just before sunset). The following behaviours were recorded: stationary active (self-grooming, eating or drinking), positive or negative interaction (between two rabbits or more: in contact, allogrooming, sniffing, fighting), moving (walking, jumping, running), standing up (on their hind legs with their front legs raised). The results are expressed as the percentage of total observations. The occurrence of inactivity (immobile rabbits) was also recorded.

Statistical analysis

All analyses were performed using R statistical software version 4.0.3 (R Core Team, 2020).

For each variable detailed below, a compound symmetry covariance structure of the R matrix was used. Live weight on days 0 and 42 of the experiment and average daily gain between days 0 and 42 were analysed with a mixed model. The model included outdoor access, the stocking density, and their interaction as fixed effects and the pens as random effects. Mortality between days 0 and 42, a binary trait (0 = dead and 1 = alive), was analysed using a

logistic regression model with the same model. Herbage height data were analysed using a series of three independent linear models on each measurement day. The stocking density of the Y groups (two rates: H and L) was used as the explanatory variable for all three models. Spatial distribution data were analysed using a comparative proportion test. Serial pairwise independent *posthoc* proportion tests were used to compare outdoor access (two levels: Y and N) or the stocking density (two rates: H and L). The proportions represented the total number of rabbits observed at each place (data aggregated over the 36 observation points) over the total number of possible observations. Behavioural data were analysed with a mixed model. Two independent models were constructed. The first model included only rabbits with access to paddocks (YH and YL) and included the observation site (two levels: pens or paddocks), the time of day (morning, afternoon, evening), the stocking density (H or L) and their interaction as fixed effects and pen as a random effect. The second model for behaviour data included all groups (four levels: YH, YL, NH and NL), but only the data on the behaviour of rabbits present in the pens during video recordings were analysed. The second model included outdoor access, the stocking density, the time of day (morning, afternoon, evening) and their interactions as fixed effects and pen as the random effect.

The new environment and novel object test data were analysed with a general linear model based on a binomial distribution. The model for the new environment test included the period of observation (four intervals: 0–5, 5–10, 10–15 and 15–20 min), the observation days (days 0, 20 and 31), the stocking density, the sex (male or female) and their interactions. When access to paddocks was considered as the novel environment, this effect was not included in the model (see above). The model for the novel object test included outdoor access, the stocking density, the period of observation (three intervals: 0–3, 3–7 and 7–10 min), the observation days (days 4 and 33; corresponding to the rabbits' ages of 35 and 64 days), the sex, and their interaction. The model used for the human approach test was the same as the model used in the novel object test, except for the period of observation.

Results

Environmental conditions and herbage allowance

The average indoor and outdoor temperatures were 18.2 °C (min 7.5 °C and max 31.5 °C) and 13.9 °C (min 1.3 °C and max 24.7 °C), respectively, throughout the experiment. Temperatures near the exit hatches averaged 21.1 °C (min 8.4 °C and max 30.9 °C) for the Y groups and 22.2 °C (min 10.4 °C and max 32.4 °C) for the N groups ($P < 0.05$). Temperatures near the central aisle averaged 21.1 °C (min 8.2 °C and max 29.6 °C) for the Y groups and 21.7 °C (min 8.2 °C and max 32.7 °C) for the N groups ($P = 0.24$). Outside wind speed averaged 2.8 m/s and rainfall was 2.4 mm (min 0 mm/d and max 24.5 mm/d).

The average overall herbage height was 11.6 ± 5.1 cm at the beginning of the period of outdoor access (Table 1). The botanical composition of pasture was heterogeneous among the plots as the proportion of grass ranged from 35% to 54% and the proportion of legumes ranged from 46% to 61%. The chemical composition (pooled samples) of the meadow was 13.4% DM, 3.8% of CP, 5.5% of NDF, 2.7% of ADF, 0.6% of lignin and 2.0% of ash on a raw basis. Between day –1 and day 27, the respective proportion of grass and legumes respectively decreased from 35.0% to 30.4% and from 61.0% to 27.6% for the YL group, and from 54.0% to 9.5% and from 46.0% to 1.3% for the YH group. Rabbits in the YH group consumed all the herbage in the pasture in 17 days after having access to the paddocks while rabbits in the YL group needed 10 additional days

Table 1

Herbage height (cm) and percentage of grass, legumes, and bare ground in the pastures available to rabbits having access to a paddock housed at a low (YL) or high (YH) stocking density inside pens and the day on which the measurements were taken (experimental days: –1, 27 and 41).

Item	Groups		P-value
	YL	YH	
Herbage height (cm) [†]			
Day –1	11.4 ± 0.49	12.2 ± 0.49	0.28
Day 27	2.3 ± 0.16	2.1 ± 0.23	0.42
Day 41	2.3 ^a ± 0.22	1.3 ^b ± 0.39	<0.05
Grass (%) [‡]			
Day –1	35.0 ^a	54.0 ^b	<0.05
Day 27	30.4 ^a	9.5 ^b	<0.05
Day 41	38.0 ^a	12.0 ^b	<0.05
Legumes (%)			
Day –1	61.0	46.0	0.05
Day 27	27.6 ^a	1.3 ^b	<0.05
Day 41	0.0	0.0	
Bare ground (%)			
Day –1	4.0	0.0	0.10
Day 27	42.0 ^a	89.2 ^b	<0.05
Day 41	62.0 ^a	88.0 ^b	<0.05

[†] Means for herbage height do not include the percentage of bare ground (Day –1: 96 points for YL and 100 points for YH; Day 27: 70 points for YL and 34 for YH; Day 41: 38 points for YL and 12 points for YH).

[‡] Proportion of grass in relation to the number of measurements taken in each group (100 measurement points per group).

^{a-b} Values with different superscripts in the same row differ significantly at $P < 0.05$.

to entirely consume the herbage in the pasture with only stems remaining.

Mortality and growth traits

During the experiment, one rabbit escaped from the outdoor area and was not recaptured. This animal was included in the animal losses as dead. The mortality rate from day 0 to day 42 reached 5% and no effect of stocking density nor interaction between outdoor access and stocking density was observed. Mortality tended to be higher in groups that had outdoor access (7% in Y and 3% in N groups, $P = 0.06$; Table 2). Rabbits had a good general health status throughout the experiment (98% of live rabbits were diagnosed as healthy at day 42) regardless of the experimental group.

The live weight of rabbits was similar between groups at day 0 (on average 879 ± 98 g; Table 2 and Supplementary Fig. S1). The interaction between access and stocking density on live weight and growth rate was not significant. At day 42, the weight of the rabbits was higher in the N than in the Y groups (+134 g; $P < 0.05$), but no differences were observed in rabbits reared at high and low stocking density ($2\,093 \pm 159$ g vs $2\,056 \pm 170$ g, respectively; $P = 0.24$). Similarly, the average daily gain between day 0 and day 42 was 13% higher in the N groups than in the Y groups (30.2 ± 2.2 vs 26.7 ± 2.7 g/d, respectively; $P < 0.05$) and was 4% higher in the H vs L groups (28.9 ± 3.0 vs 27.7 ± 2.9 g/d, respectively; $P = 0.28$).

Behavioural tests

Spatial distribution

Throughout the observation period, from experimental days 6 to 41, at observation times, rabbits were more frequently seen under the platforms (Table 3). The proportion of rabbits in the L groups seen under the platforms was higher than the proportion in H groups. Between experimental days 6 to 17, it was 8 percentage points higher in YL than in YH and 10 percentage points higher in NL than in NH groups ($P < 0.05$). Between experimental days 33

Table 2

Growth and mortality of rabbits according to outdoor access (Y: yes or N: no) and stocking density inside pens (H: high or L: low).

Item	Outdoor Access (A)		Stocking density (S)		P-value [†]		
	Y	N	L	H	A	S	A × S
N° of rabbits at day 0	150	149	100	199			
Live weight (g) at day 0	883 (±104)	874 (±93)	886 (±105)	875 (±95)	0.98	0.47	0.12
N° of rabbits at day 42	139	145	95	189			
Live weight (g) at day 42	2011 (±157)	2145 (±141)	2056 (±170)	2093 (±159)	<0.05	0.24	0.43
ADG [‡] (g/d) from day 0 to day 42	26.7 (±2.7)	30.2 (±2.2)	27.7 (±2.9)	28.9 (±3.0)	<0.05	0.28	0.91
Mortality (%)	7.3	2.7	5.0	5.0	0.06	0.97	0.41

[†] Interaction between access and stocking density was not significant.[‡] Average daily gain.**Table 3**

Spatial distribution of rabbits (expressed as frequency of observations) according to outdoor access (Y: yes or N: no) and stocking density inside pens (L: low or H: high) between day 6 and day 41.

Item	Groups				P-value	
	YL	YH	NL	NH	YL-YH	NL-NH
Days 6–17						
Floor between the platforms (%)	17	27	33	42	<0.05	<0.05
Floor under the platforms (%)	54	46	62	52	<0.05	<0.05
Platform area (%)	5	6	5	7	0.40	0.20
A1 (0–2 m from hatch) (%)	14	11			0.30	
A2 (2–8 m from hatch) (%)	11	9			0.30	
Days 19–31						
Floor between the platforms (%)	18	25	33	41	<0.05	<0.05
Floor under the platforms (%)	47	45	56	43	0.50	<0.05
Platform area (%)	7	6	12	16	0.40	<0.05
A1 (%)	12	14			0.30	
A2 (%)	16	10			<0.05	
Days 33–41						
Floor between the platforms (%)	16	23	36	35	<0.05	0.90
Floor under the platforms (%)	57	42	52	43	<0.05	<0.05
Platform area (%)	4	9	12	22	<0.05	<0.05
A1 (%)	9	20			<0.05	
A2 (%)	14	6			<0.05	

to 41, the values were 57% vs 42% in YL vs YH groups, and 52% vs 43% in NL vs NH groups ($P < 0.05$).

Conversely, the proportion of rabbits seen on the platforms in the H groups was higher than the proportion in the L groups (16% vs 12% and 22% vs 12% in NH vs NL between days 19 and 31, and between days 33 and 41 respectively; $P < 0.05$). In the same way, the proportion of rabbits seen in the floor area between platforms was also higher in the H groups than in the L groups (26% vs 19% in the YH vs the YL group from day 6 to day 41 and 26% vs 17% in NH vs NL from day 6 to day 31, $P < 0.05$). In the Y groups, the second most used area was the paddock, an average of 21% of rabbits were seen in the paddocks between experimental days 6 and 41. The proportion of rabbits in the A2 area was higher in YL between days 19 and 31 (16% vs 10% in YL vs YH, $P < 0.05$) and between days 33 and 41 (14% vs 6% for YL vs YH; $P < 0.05$). However, the opposite was the case in the A1 area, where the proportion of rabbits in the YH group was higher (20% vs 9% in YH vs YL between days 33 and 41; $P < 0.05$). Overall, no pronounced differences in spatial distribution were observed at the different times of day (Data not shown).

New environment test

The latency time for rabbits to access the pasture increased with the day of observation (600 ± 89 s at day 31 vs 50 ± 68 s at day 3; $P < 0.001$; [Supplementary Fig. S2](#)).

[Fig. 2](#) shows the percentage of rabbits observed in the paddocks during the new environment test. Only interactions between stocking density and day of observation, minute in the test and observation day, and stocking density and minute in the test were significant ($P < 0.001$). More rabbits in the L groups were counted in the paddocks at day 3 than at day 20 or at day 31 (37% vs 30% vs 11%; $P < 0.001$) while fewer rabbits in the H groups went outside on day 3 than on day 20 (13% vs 19%, respectively; $P < 0.001$), but more rabbits in the H groups went outside on day 31 (13% vs 9%, respectively; $P < 0.001$). The percentage of rabbits in the paddocks 5 min after the exit hatch opened decreased with day of observation (15%, 5% and 1% at day 3, day 20 and day 31, respectively; $P < 0.001$). 20 min later, the percentage was higher on day 20 than on day 3 (51% vs 36%, respectively; $P < 0.001$), but lower on day 31 than on day 3 (26% vs 36%, respectively; $P = 0.25$). Females were more frequently observed in the paddocks than males ($P < 0.001$) on day 3 (32% vs 18%), on day 20 (30% vs 18%), and on day 31 (13% vs 7%).

Human approach test

Interactions between the day of observation and access and the day of observation and the stocking density for the first contact latency were significant ($P < 0.001$; [Supplementary Fig. S3](#)). The average time of first contact with the operator's hand was 8.0 ± 5.0 s on day 5 regardless of the experimental group. On day

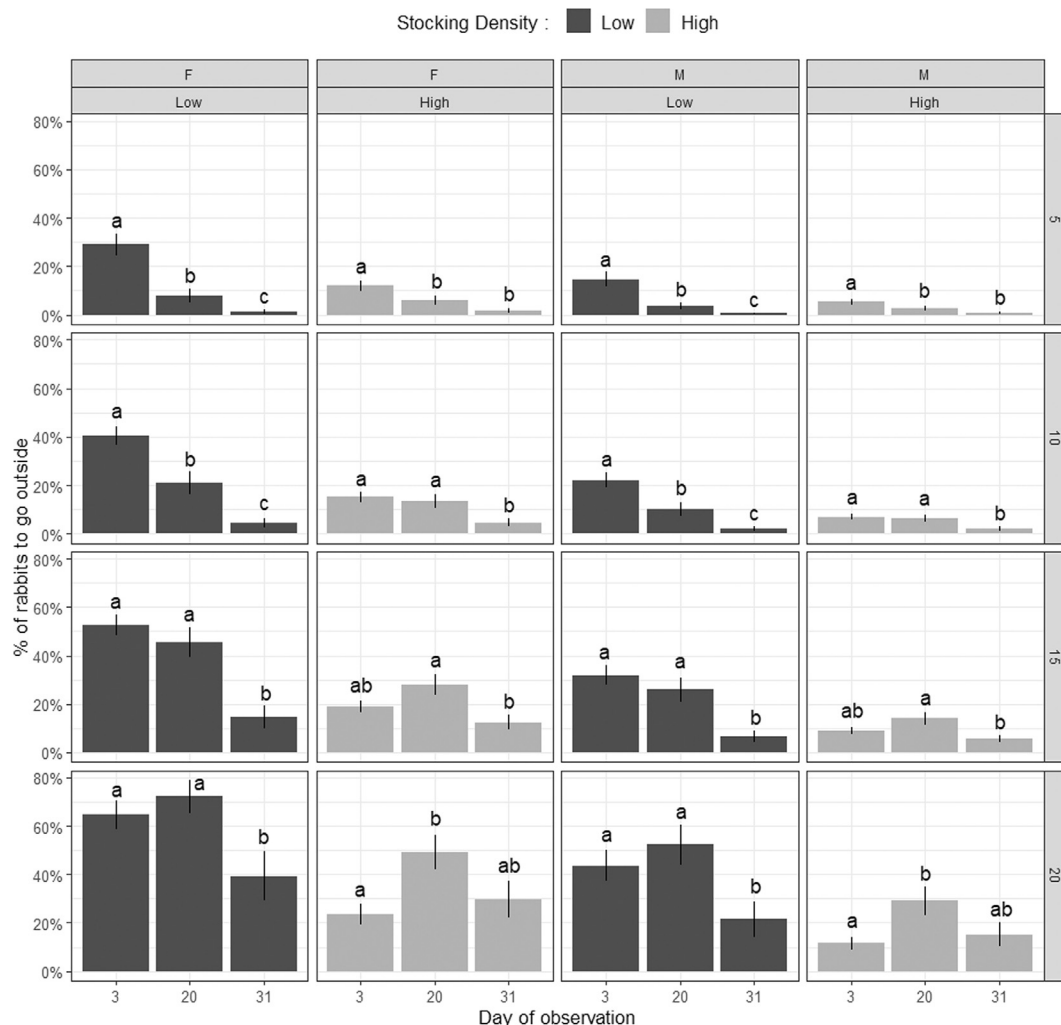


Fig. 2. Percentage of rabbits observed in the paddocks during the new environment test as a function of the observation day (OD: 3, 20 or 31, corresponding to ages 30, 51 or 62 days), the sex (S: F or M), the minute in the test (MT: 5, 10, 15 or 20 min) and the stocking density (SD: Low or High). For each sex, bars in each panel (sex and minute) with different letters (a, b, c) differ at $P < 0.05$. The day of observation and the two-way interactions between OD \times MT, OD \times SD, and MT \times S were all significant ($P < 0.001$), while the single effects of stocking density, sex and minute in the test were not significant.

32, latency to first contact was higher in animals in the L groups than in animals in the H group (81.0 ± 20.0 s vs 5.0 ± 3.0 s, respectively; $P < 0.001$).

Fig. 3 shows the percentage of rabbits that touched the operator's hand during the test. Interaction between access and density was significant ($P < 0.05$). At day 5, 44% of rabbits in the NL group touched the operator's hand compared to 18% of rabbits in the NH group ($P < 0.05$) while the percentage was similar in the YL, YH and NH groups. At day 32, the percentage of rabbits that touched the operator's hand was not significantly different among groups. The pattern between groups was very similar at days 5 and 32, with no differences depending on the observation day ($P = 0.52$). Sex had no significant effect on the percentage of rabbits that touched the operator's hand during the test ($P = 0.84$).

Novel object test

Only the interaction between day of observation and access was significant for the first contact latency ($P < 0.001$; Supplementary Fig. S4). Latency to first contact was six times longer in Y groups at day 33 than at day 4 (46.0 ± 33.0 s vs 7.0 ± 8.0 s, respectively; $P < 0.001$). At day 33, rabbits in the Y groups took longer time before their first contact than in the N groups (46.0 ± 33.0 s vs 4.0 ± 3.0 s; $P < 0.05$). Rabbits in the H groups took less time to first

contact than those in the L groups (4.0 ± 2.0 s vs 10.0 ± 7.0 s at day 35 and 10 ± 9 s vs 40 ± 39 s at day 64; $P < 0.001$).

Duration of the test had no effect on the percentage of rabbits that approached the object ($P > 0.05$). In Fig. 4, we thus only show the average percentage of rabbits that approached the new object according to sex, the day of observation, and the group. The interaction between outdoor access and stocking density was significant ($P < 0.05$) as was the interaction between day of observation and sex ($P < 0.001$). Independently of the sex, the percentage of rabbits that approached the object was higher in the NL group than in YL (30.9 vs 15.5% , respectively; $P < 0.05$). The percentage of females to approach the novel object was higher on day 4 than on day 33 (on average 20% vs 15% ; $P > 0.05$) whereas the percentage of males approaching the novel object was lower on day 4 than on day 33 (on average 19% vs 29% ; $P < 0.05$).

Comparison of the behaviour of rabbits in the groups with access to paddocks (Y groups only)

The rabbits' behaviour differed depending on whether the animals were in the paddocks or in the pens (Table 4). Interactions between the time of day and the observation site and the stocking density were only significant for activity at day 26 ($P < 0.05$). Concerning activity, there was also an interaction between the obser-

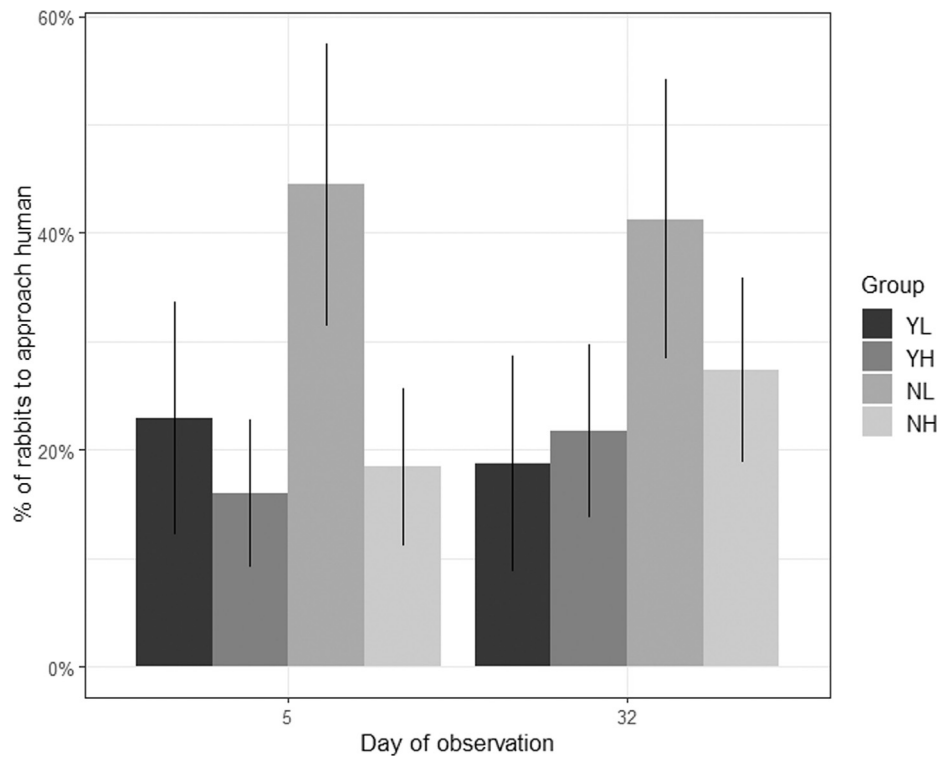


Fig. 3. Percentage of rabbits that touched the operator's hand in the human approach test as a function of the observation day (day 5 or 32, corresponding to ages 36 and 63 days), outdoor access (Y: yes, or N: no) and stocking density (H: high or L: low). The interaction between outdoor access and stocking density was significant ($P < 0.05$).

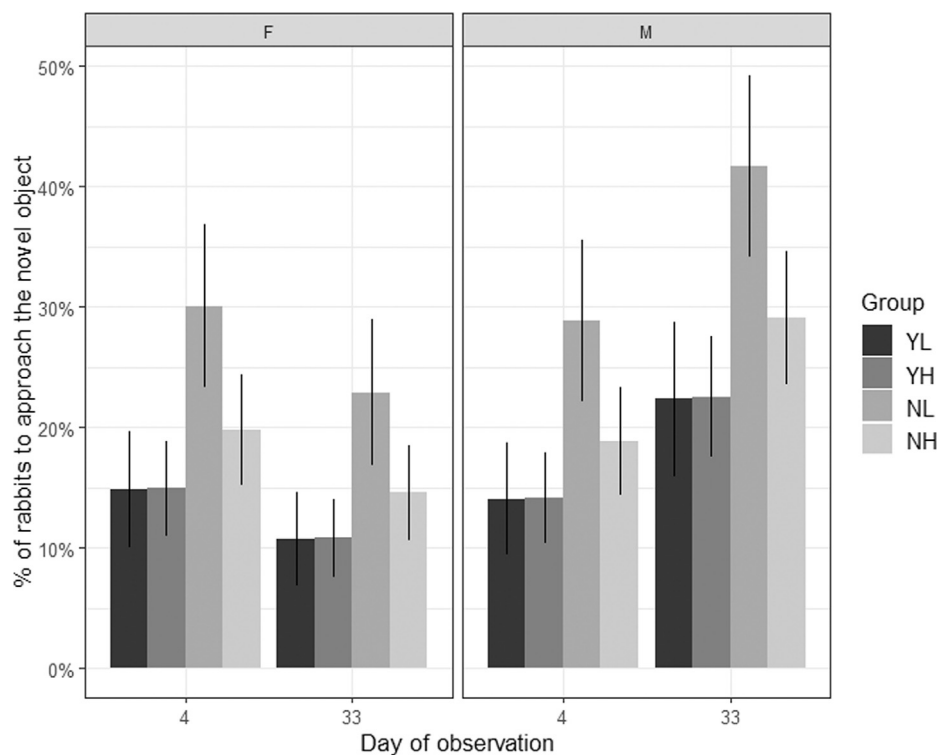


Fig. 4. Percentage of rabbits that touched the object during the novel object test as a function of the group (YL, YH, NL, NH), the sex (F or M) and the day of observation (day 4 or 33, corresponding to ages 35 and 64 days). The effect of outdoor access and stocking density was significant ($P < 0.05$) as was the effect of the day of observation and the sex ($P < 0.001$). Abbreviations: Y = yes to outdoor access, N = no to outdoor access, H = High stocking density, L = Low high stocking rate.

vation site and the time of day ($P < 0.05$). In the pens, rabbits in the L group were more active in the morning than in the afternoon (20.1% vs 15.0%; $P < 0.001$) while rabbits in the H group were more

active in the afternoon (15.7% vs 10.9%; $P < 0.001$). Concerning locomotion, only the observation site (paddocks or pens) was statistically significant ($P < 0.05$). Locomotion was more frequent in

Table 4

Occurrence of behaviours of rabbits according to the time of day (morning, afternoon, early evening), the observation site (paddocks or pens) and the stocking density (high or low) at days 26 and 40.

Item	Morning				Afternoon				Early evening				P-value [†]		
	Outside		Inside		Outside		Inside		Outside		Inside		Time	Site	Stocking density
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low			
Day 26															
Rabbits (n)	93	47	93	47	93	47	93	47	93	47	93	47			
Activity (%)	44.1	44.5	10.9	20.1	6.3	37.1	15.7	15.0	50.0	52.5	23.1	25.1	<0.05	<0.001	0.10
Interaction (%)	2.4	5.3	4.3	3.3	0.7	1.7	2.9	3.1	2.7	4.0	4.0	3.8	0.09	0.26	0.38
Moving (%)	17.1	15.3	2.4	11.8	4.9	22.6	6.2	6.0	21.3	22.6	10.2	11.2	0.22	<0.05	0.27
Standing up (%)	4.1	4.2	0.4	6.6	0.8	8.0	0.1	0.2	4.6	6.5	3.3	1.7	0.67	0.15	0.30
Inactivity (%)	32.3	30.7	82.0	58.2	87.3	30.6	75.1	75.7	21.4	14.4	59.4	58.2	<0.05	<0.001	0.15
Day 40															
Rabbits (n)	93	46	93	46	93	46	93	46	93	46	93	46			
Activity (%)	52.0	51.3	29.0	30.7	31.3	19.4	8.0	8.2	15.6	32.8	20.8	30.4	<0.05	<0.05	0.72
Interaction (%)	12.5	12.6	4.4	6.5	2.0	21.6	1.8	2.7	3.8	10.6	3.0	3.4	<0.05	<0.05	0.06
Moving (%)	23.0	24.5	9.0	13.7	36.1	14.0	2.4	1.6	10.5	25.8	8.6	16.4	0.92	<0.05	0.85
Standing up (%)	2.2	5.1	3.6	8.1	0.1	0.0	0.2	0.1	0.3	1.8	1.1	6.2	<0.05	0.22	0.36
Inactivity (%)	10.3	6.5	54.0	41.0	30.5	45.0	87.6	87.4	69.8	29.0	66.5	43.4	0.06	<0.05	0.37

[†] Interactions between time of day, observation site and stocking density were not significant except activity at day 26 ($P < 0.05$).

the paddocks than inside the pens (on average, 17.3% vs 9.5% respectively; $P < 0.05$). The percentage of inactive rabbits in the pens was higher than the percentage observed in the paddocks (68.1 vs 36.1%, respectively; $P < 0.001$). The effect of the time of day on the percentage of inactive rabbits was significant. The percentage of inactive rabbits was higher in the morning and afternoon than in the early evening (50.8%, 67.2% and 30.4%, respectively, $P < 0.05$).

At day 40, more activity was observed in the morning (40.8%) than in the afternoon (14.3%) ($P < 0.001$) and in the evening (24.9%) ($P < 0.05$). Activity was more frequent in the paddocks than in the pens (32.1% vs 21.2%; $P < 0.05$). Locomotion was more frequent in the paddocks than in the pens (on average, 22.3% vs 8.6% respectively; $P < 0.05$). There were more interactions among animals in the paddocks than in the pens (8.7% vs 3.6%; $P < 0.05$). The percentage of standing-up rabbits was higher in the morning than in the afternoon (4.7 vs 0.1%, respectively; $P < 0.05$). On day 26, the percentage of inactive rabbits was higher in the pens than in the paddocks (63.3% vs 28.1%, respectively; $P < 0.05$).

Comparison of rabbit behaviour in the pens according to outdoor access, stocking density, and the time of day assessed at two ages

Behaviour in the pens was only affected by the time of day (morning, afternoon, or evening; Table 5). At day 26, only the percentage of active rabbits differed. Rabbits were more active in the early evening than in the morning and afternoon (20.7% vs 14.5% and 14.4%, respectively; $P < 0.05$). Inactivity was the most common behaviour inside the pens, with no differences among experimental groups.

At day 40, all behaviours were affected by the time of day. Rabbits were more active in the morning and in the early evening than in the afternoon (25.7% vs 7.0%; $P < 0.05$ and 19.4% vs 7.0%; $P = 0.07$). Interaction among rabbits was more frequently observed in the morning than in the afternoon and in the early evening (5.6% vs 2.2% and 2.9%; $P < 0.05$). Locomotion was more frequent in the morning and in the early evening than in the afternoon (10.1% and 9.6% vs 2.7%; $P < 0.05$). Standing-up behaviour was more frequent in the morning than in the afternoon (4.2% vs 0.1%; $P < 0.05$). Inactivity was higher in the afternoon than in the morn-

Table 5

Occurrence of behaviour of rabbits inside the pens according to outdoor access: yes (Y) or no (N), stocking density (low or high) and the time of day (morning, afternoon, early evening) at days 26 and 40.

Item	Outdoor Access (A)		Stocking density (S)		Daytime (D)			P-value [†]		
	Y	N	Low	High	Morning	Afternoon	Early evening	A	S	D
	Day 26									
Rabbits (n)	140	147	96	191	287	287	287			
Activity (%)	18.3	15.2	16.6	16.8	14.5 ^a	14.4 ^a	20.7 ^b	0.11	0.89	<0.05
Interaction (%)	3.6	4.2	3.5	4.2	4.1	2.9	4.7	0.37	0.29	0.08
Moving (%)	7.9	5.9	8.0	5.9	5.4	6.3	8.7	0.29	0.29	0.17
Standing up (%)	2.0	1.6	2.1	1.5	2.4	0.5	2.4	0.76	0.69	0.18
Inactivity (%)	68.2	73.1	69.8	71.6	73.6	75.9	63.5	0.27	0.17	0.06
Day 40										
Rabbits (n)	139	145	94	190	284	284	284			
Activity (%)	21.2	13.5	17.8	16.9	25.7 ^b	7.0 ^a	19.4 ^{ab}	0.16	0.85	<0.05
Interaction (%)	3.6	3.5	3.9	3.2	5.6 ^b	2.2 ^a	2.9 ^a	0.90	0.50	<0.05
Moving (%)	8.6	6.3	8.8	6.1	10.1 ^b	2.7 ^a	9.6 ^b	0.37	0.31	<0.05
Standing up (%)	3.2	1.3	3.1	1.4	4.2 ^a	0.1 ^b	2.4 ^{ab}	0.28	0.33	<0.05
Inactivity (%)	63.4	75.4	66.4	72.4	54.4 ^a	88.0 ^b	65.7 ^{ab}	0.23	0.52	<0.05

[†] Interaction between outdoor access (A) and stocking density (S) and time of day (D) was not significant ($P > 0.05$). Interaction between outdoor access (A) and stocking density (S); outdoor access (A) and time of day (D); stocking density (S) and time of day (D) was not significant ($P > 0.05$).

^{a-b} Values with different superscripts in the same row differ significantly at $P < 0.05$.

ing and in the early evening (88 vs 54.4 and 65.7%, respectively; $P < 0.05$).

Discussion

In the light of our main results, we focus our discussion on the main aspects concerning use of pasture and the behaviour of rabbits. We also discuss our results concerning the growth and mortality of weaned rabbits raised in a system allowing outdoor access.

Pasture usage

After the rabbits were given access to outdoors, herbage in the pastures was completely consumed in 17 days by rabbits in the YH group and in 27 days by rabbits in the YL group. Only bare ground, stems, and some patches of very short dry grass remained. This result can be explained by the limited outdoor space provided to the animals at the stocking density applied (23 m² for 25 or 50 rabbits in the YL and YH groups, respectively), compared to the herbage intake capacity of rabbits. Legendre et al. (2019) calculated that rabbits can ingest a maximum of 75 g of DM of herbage per kg^{0.75} (roughly 0.6 m²/day/rabbit of pasture area to cover their requirements). These values are far above those applied in our experiment.

Although we cannot precisely describe the botanical characteristics of the herbage consumed in the first 2 weeks of outdoor access (overall height reduction of 81.5%), the percentage of legumes that disappeared in this period was 55% in the YL group and 97.2% in the YH group, while the observed reduction in grass was 15% and 82.4% in the YL and YH groups, respectively. Based on these rough approximations, we can conclude that legumes were preferred over grass, which can be explained by the selective feeding behaviour of rabbits. According to Gidenne et al. (2015), rabbits prefer leaves to stems, and green and tender plant parts to dry parts.

Growth traits and mortality

Average daily weight gain of all examined rabbits during the whole growing period (around 28.5 g/day) was low compared to the gain recorded indoors with pellet feed (43.6 g/day; Read et al., 2016). The difference is mostly explained by our feeding strategy but also by the genotype used in the experiment. Rabbits originated from females belonged to a pure strain of INRA 1777 selected for maternal traits and not from a terminal cross with males selected for growth rate, as was the case in Read et al. (2016). In a recent trial in our laboratory, the observed ADG of purebred INRA 1777 growing rabbits was 38.5 g/d (unpublished data). Moreover, the rabbits in the present study were subjected to a feed restriction throughout the experimental period, whereas it is generally recommended to provide *ad libitum* access to the feed in the last 2 weeks of the growing period (Gidenne et al., 2009). *Ad libitum* access to feed was used in both previous trials (Read et al. 2016 and unpublished trial). In the present study, ADG was 3.5 g/day lower in rabbits with access to a paddock than in rabbits with no access. Since the pelleted commercial feed was entirely consumed, the nutritional contribution of grazing was negligible (60 vs 128 g/DM/rabbit for YH and YL, respectively, from day 6 to day 42). We thus hypothesise that the increased physical activity of rabbits in the Y group compared to that in the N group explains their lower ADG. This result agrees with the results of Pinheiro et al. (2012) and of Loponte et al. (2018).

Overall mortality was low (5.0%). This value is comparable to the mortality rate observed by Read et al. (2016) in an indoor pro-

duction system and was not significantly increased by access to a paddock or by the stocking density.

Behaviour

Spatial distribution

The size of groups had an effect on the spatial distribution of rabbits in the pens. Rabbits in the L groups were more often found under the platforms while rabbits in the H groups were distributed all over the floor of the pen. The reduced space available per rabbit in the H groups may be the explanation. The animals were obliged to occupy the whole floor area.

Even if platforms allow rabbits to increase their locomotion (de Jong et al., 2011), only 9% of rabbits were found on the platforms (average throughout the experimental period, all groups combined). However, and only in the case of rabbits in the NH group, the use of platforms increased with time (7% from days 6 to 17, 16% from days 19 to 31, and 22% from days 33 to 41). The limited space (2 m² excluding platform area) combined with the large number of rabbits could explain the observed spatial distribution. The pattern was not reproduced by rabbits with access to the grazing area (YH ~ 7.5% vs NH ~ 19.0%; statistical analyses were not performed). Access to paddocks provided rabbits with more space, and the paddock was preferred to the platform. The area under the platform was preferred to the other areas (including outdoor access). Overall, 51% of the rabbits observed were found on the floor below the platforms (daytime observations only). Kolb (1986) demonstrated that wild rabbits tend to remain in covered areas and burrow during daytime. One can therefore hypothesise that the area under the platform mimics a shelter for rabbits (Lombardi et al., 2007; Beja et al., 2007).

We observed no pronounced differences between the time of day (morning or afternoon) on the spatial distribution of rabbits (data not shown). A wiser choice of observation times would have been preferable. It is known that wild rabbits spent between 6.5 and 13.3 h outside their burrows (Mykytowycz and Rowley, 1958) and their exploratory behaviour occurs during the scotophase (Jilge and Stähle, 1984; Piccione et al., 2010). Therefore, the observation times used in our experiment mainly reflect rabbits' period of inactivity (use of the area under the platforms) and do not properly reflect the use of the paddocks. In future experiments, we recommend observing the rabbits at dawn and dusk rather than in the morning and afternoon in order to evaluate rabbit behaviour during their periods of peak activity.

Reactivity

Rabbits were slower (regarding latency time) and fewer to go outside in the new environment test with increasing age. Trocino et al. (2018) reported a decrease in rabbit's motivation to explore with increasing age. Here, we cannot strictly conclude on the effect of age because the same rabbits were not tested on each observation day. In addition to the possible age effect, the lack of grass and legumes available in the pastures (day 23 for YH group and day 33 for YL group) could explain the reduced motivation and the higher latency time for rabbits to go outside.

We observed no effect of stocking density or of access to a paddock in the latency time for the first contact to human at day 5 (Supplementary Fig. S3). This may be linked to the fact that while in the nest, rabbits are handled daily, thus reducing their fear of humans (Kersten et al., 1989; Csatádi et al., 2005). By the end of the experiment, rabbits in the H groups responded more quickly to novelty than rabbits in the L group both in approaching the human operator and the novel object. According to Trocino et al. (2018), high stocking density leads to involuntary approaches.

Independently of the observation day, the proportion of rabbits to touch a human was higher in the N groups (Fig. 3). Firstly, we

can hypothesise that outdoor access is a stimulus in itself (space, natural light, sounds, odours, food resources, etc.) reducing the rabbits' interest in humans (even when they were placed in the pen for the test). They were also less responsive to the first contact latency during the novel object test, and were fewer to approach it (Supplementary Fig. S4 and Fig. 4). In the wild, rabbits are easy prey. We can also hypothesise that in this study, their fear could have increased with external stimuli. Indeed, we frequently observed that the rabbits in the pasture took an alert posture when a plane flew overhead. Once the novel object was hung above them, they could have transliterated it with the planes. In our conditions, the test of the novel object was limited by being used to assess the fear of the group and not of individual rabbits.

In rodents, Archer (1975) showed that males tended to stay stiller than females, while females showed a less emotional reaction to a new environment. In rabbits, males are more alert, cautious, and territorial than females (Coureaud et al., 2015). Here, we observed a similar pattern. More females went outside than males in the new environment test (Fig. 2).

Variety of behaviours

Rabbits in the pastures were more *active*, *moved more*, and were less *inactive* than rabbits in the pens (Table 4). The bigger area available to each rabbit and the outdoor area itself, which offered several stimuli, allow rabbits to express a more diversified behavioural repertoire (D'Agata et al., 2009; Mugnai et al., 2014).

Rabbits were less *inactive* in the morning and in the evening (Table 5). Prud'hon et al. (1975) observed that rabbits adapted their behaviour to the predator pressure by being more active in the early morning, and in the evening. Princz et al. (2008) confirmed this observation and showed that rabbits were less active between 1100 h and 1700 h.

In our study, regardless of the stocking density and for rabbits observed inside pens, a high proportion of *inactive* rabbits was observed all day long. Morisse and Maurice (1997) showed that high stocking density (>16 rabbits/m²) led rabbits to spend more time inactive than low stocking density (12 rabbits/m²). Luzi et al. (2003) reported that rabbits lacking stimuli are less prone to activity.

Conclusion

Present results showed that the outdoor access reduced the growth of rabbits and tended to increase their mortality rate regardless of the stocking density. However, both growth rate and survival were acceptable with respect to the genotype and feeding strategy used in our experiment. When they had access to pasture, rabbits were more active than indoor and displayed a wider range of behaviours (hopping, standing up, grazing, positive interactions, etc.), which is in favour of animal welfare. However, emotions of animals need to be better characterised. If grazing seems to meet the expectations of rabbits, the outdoor access may increase alertness and/or fear when they are outside. The total consumption of pasture biomass may hinder subsequent grass regrowth. Therefore, further studies concerning the optimum paddock area and the management of the access time to the outdoor area seem necessary to improve the functioning of such a farming system.

Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2021.100334>.

Ethics approval

The French Committee (number 115) for Ethics, Science and Animal Health approved all the experimental interventions on animals. The committee followed the provisions laid down in articles R.214-87 to R.214-126 of the French Rural Code. The experiment permit number is 16330-20180727162112112.

Data and model availability statement

None of the data were deposited in an official repository. The access rights to data are available upon request.

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Declaration of interest

The authors declare no conflict of interest of any sort.

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