Effect of outdoor grazing area size and genotype on space and pasture use, behaviour, health, and growth traits of weaned rabbits

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Research article

Effect of outdoor grazing area size and genotype on space and pasture use, behaviour, health, and growth traits of weaned rabbits

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A B S T R A C T

Providing rabbits with outdoor access allows them to express a large repertoire of behaviours and addresses societal expectations surrounding animal welfare in livestock systems. The aim of this work was to study rabbit growth, health, and behaviour according to genetic type and pasture area size. We distributed 192 weaned rabbits into two groups with different pasture sizes, i.e., a large pasture (**LP**): 60 m^2 (n = 4 × 24) and a small pasture (**SP**): 30 m^2 (n = 4 × 24). Each group contained half Californian 1001 × New Zealand 1777 rabbits (**1001**) and half PS119 × New Zealand 1777 rabbits (**PS119**). Rabbits were reared from 31 to 73 days of age. Rabbit growth and morbidity were measured individually weekly for 36 days (from 31 to 67 days of age). The rabbits' behaviours were assessed three times a day on days 44, 58, and 70 by a direct visual scan, and the use of space was evaluated six times a week based on the rabbits' spatial distributions. The available biomass was evaluated on days 35, 50, and 73. Finally, the amount of corticosterone was measured in hair samples from 18 rabbits of each genotype in LP and SP on day 72. PS119 rabbits were heavier than 1001 rabbits on day 67 (2 444 vs 2 113 g, respectively; $P < 0.05$) but we observed no effect of genotype on mortality or morbidity. The animals expressed a large variety of specific behaviours on pastures, with grazing and resting being predominant. We found a lower level of corticosterone in PS119 rabbits than in 1001 rabbits (2.19 vs 6.34 pg per mg of hair, respectively; $P < 0.05$). LP pastures offered herbage until the end of the fattening period, enabling the rabbits to express grazing behaviour until the end of the experiment, and grazing behaviour was more frequent in LP-group rabbits (25.4 vs 21.5% of occurrences in LP vs SP; $P < 0.05$). This study found that a pasture of 60 m^2 for 24 rabbits enables the animals to express grazing for the entire fattening period. The rabbit genotype was found to influence some of their behaviour.

Implications

Outdoor access allows rabbits to express a large repertoire of behaviours and addresses social expectations surrounding animal welfare in livestock systems. The alternative of caged systems tested here provides rabbits with different genotypes access to pastures differing in surface area. The larger pastures enable the rabbits to graze consistently throughout the growing period and to better accommodate their behavioural needs. As rabbits farmed outdoors are also exposed to external stressors, we tested two different genotypes to find out how they coped. We found both pasture size- and genotype-related differences in the expression of behaviours, and genotype-related differences in growth and stress.

Specification table

Subject	Welfare
Type of data	Tables, Figures, Images
How data were acquired	Scale, direct observation of animals, microscope, ELISA immunoassay kit, data logger, statistical analysis with R 4.0.3 software
Data format	Raw and pretreated
Parameters for data collection	We collected herbage measures, and we observed the expression of behaviours of rabbits under non-rainy and non-windy conditions. For the other measures, there were no specific conditions.

(*continued on next page*)

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Description of data collection	Live weight: once a week, individually Behaviours: scan-sampling method, three times a day at three ages Corticosterone: at 72 days, a patch of hair was shaved from 80 rabbits between the shoulders, and corticosterone was extracted from the hair samples Spatial distribution: animals were counted in different areas in the pens and in the pastures three times a week and twice a day
Data source location	Institution: INRAE Town/Region: Pompertuzat, Occitanie, France
Data accessibility	Repository name: Mendeley Data identification number: https://data.mendeley.com/datasets/v22g4c8454/1
Related research article	No research article is related to this article

Introduction

Industrial rabbit production systems are attracting mounting criticism in Europe relating to the use of cages, the small amount of space available per rabbit, and the limited range of expression of their specific behavioural repertoire. Rabbit husbandry is of particular concern, as more than 90% of meat rabbits farmed in the EU live in wire cages (Szendrö et al., 2019). It has now long been proven that cage housing is not suitable for rabbit welfare, as it restricts locomotion (Lehmann, 1987), reduces the expression of several specific behaviours such as social activity (Trocino and Xiccato, 2006), and has adverse effects on health (spinal column deformation and pododermatitis; Drescher, 1992; Rosell and Fernando de la Fuente, 2013). There is, therefore, an urgent need to carry out a transition to cage-free systems that provide better animal health and welfare conditions.

One possible solution is to provide rabbits with outdoor access. The foremost priority for the French citizens surveyed as part of the 'ACCEPT' project was that breeding animals should have access to the outdoors (Delanoue et al., 2018). Moreover, as rabbits are herbivores, they benefit from a grassy area to express specific behaviours such as foraging (Prebble et al., 2015) and grazing and hopping (Mykytowycz and Rowley, 1958). Organic farming specifications impose at least 0.5 m^2 of outdoor area per animal for rabbits raised in a fixed building (European Group on Rabbit Nutrition, 2001). However, Fetiveau et al. (2021) showed in a non-organic fixed park system with restricted access to pellet feeding and free access to a grassy 23 m^2 pasture for 50 or 25 rabbits that the grass biomass available quickly disappeared (17–27 days, respectively), leaving mainly only bare ground at the end of the fattening period, which is detrimental to subsequent grass regrowth. The small grazing area associated with restricted access to pellet feed could explain this result. Legendre et al. (2019) described rabbit herbage intakes in organic production systems, thus providing indications regarding grazing area requirements. However, contrary to fixed housing, a mobile cage system can provide access to new grass each time the cage is moved. There is, therefore, a need to better define the appropriate outdoor area size for fixed systems to enable rabbits to express their behavioural repertoire, such as grazing, while preserving the regrowth capacity of grass throughout the experimental period.

When rabbits have outdoor access, they need to cope with a number of climatic and/or biological constraints such as parasites that can affect their growth and health (Fetiveau et al., 2021), as well as various other stressors such as noise and predators (D'Agata et al., 2009). It would, therefore, be instructive to evaluate the benefits of using different genotypes in a free-range system in terms of behaviour, growth traits, and stress factor. Here, we compared two genotypes with different origins, selection criteria, and histories of selection. Heimbürge et al. (2019) showed that corticosterone is a relevant marker of stress over a long period of time, so we determined corticosterone levels in hair as a measure of chronic stress to find out whether the genotype is a factor in terms of dealing with stress under such rearing conditions. Combined with behavioural indicators such as spatial distribution and frequency of expression of specific behaviours, these indicators can globally assess the welfare of rabbits that have access to an outdoor area.

The majority of the literature dealing with outdoor rabbits dates back to the 1950s and especially concerns wild individuals as almost all rabbits continue to be raised in confinement. There is, therefore, a need to provide updated knowledge on domestic rabbits raised outdoors. The study focused on the effects of pasture size and rabbit genotype on the growth traits, health status, behaviour, and hair corticosterone levels of weaned rabbits. We hypothesised that (i) a large pasture area would enable rabbits to graze during the entire fattening period and express a broader behavioural repertoire, preserve grass resource availability and integrity, and limit the coccidian infestation due to a lower density of droppings, and that (ii) a better-adapted genotype would better withstand the environmental conditions and exhibit faster growth traits and less stress. Given that ability to express specific behaviours and the absence of distress and disease are key prerequisites for animal welfare (The National Archives, 2012), this work also aimed to help shape the development of rabbit farming systems that are geared more to the enhancement of animal welfare.

Material and methods

Animals and experimental design

A total of 192 rabbits of both sexes (50% males and 50% females) were reared for 43 days during spring, starting from weaning (at 31 days of age) to 73 days of age. Rabbits were housed in a 30 m^2 building placed on a pasture and equipped with eight 2 m^2 roofless pens as described by Fetiveau et al. (2021). The building had been designed to be moved at each end of the growing period in order to allow better health management as well as appropriate management of biomass. Thus, in this experiment, no rabbits had previously been raised in this location. Each pen was equipped with a hayrack (W × L × H: 24 × 41 × 48 cm) placed on the opposite side of the feeder. The hayracks were 28 cm from the floor, and a wire netting (L × H: 11 × 2 cm) prevented the hay from falling. The longitudinal walls of the building had four hatches cut out to allow access to the pasture in front of each pen. The outdoor area was protected from predators by a three-wire electric fence. The pastures were enclosed by a sweet-chestnut (*Castanea sativa*) fence (H: 1.0 m). The pasture was a meadow sown in 2016 with pure fescue (*Festuca arundinacea*). The experiment took place in April 2021, and the grassland was composed of various herbaceous plants (*Festuca arundinacea, Lolium perenne*, *Trifolium repens*, *Crepis setosa*, and *Dactylis glomerata*).

Half of the rabbits were crossbred Californian 1001 × New Zealand 1777 (both INRAE genetic types selected on postweaning growth rate; **1001**) and the other half were PS119 × 1777 (semen from a commercial genetic type selected for robustness and carcass weight; https://hypharm.fr/en/products/male-ps-119/; Hypharm, 49450 Sèvremoine; **PS119**) (see Supplementary Fig. S1). At weaning, rabbits of both sexes and both genotypes were distributed into two experimental groups differing in pasture surface area, i.e., 60 m^2 (W × L: 6 × 10 m; Large pasture: **LP**) or 30 m^2 (W × L: 3 × 10 m; Small pasture: **SP**). Each group, housed in a pen having access to one pasture, contained 24 rabbits (i.e., 12 '1001' and 12 'PS119' half males and half females). The rabbits were allowed continuous access to the pasture from days 37 to 73. Throughout the experiment, the rabbits were fed *ad libitum* with a pelleted diet without coccidiostat (STABI-FIBRE, Terrya, Rignac, France; 15.2 MJ of gross energy per kg DM, 15.0% CP, 3.0% fat, 41.2% NDF, 20.8% ADF, and 6.4% ADL on a DM basis) and with sainfoin (*Onobrychis viciifolia*) hay (15.6 MJ of gross energy per kg DM, 4.4% CP, 56.6% NDF, 43.1% ADF, and 10.2% ADL on a raw basis). The animals were weighed individually once a week on days 31, 39, 46, 53, 60, and 67. The pelleted feed and hay intake per pen were measured weekly.

Ambient parameters and grassland areas

The temperature and humidity inside and outside of the building were recorded daily with a probe (Omega OM-CP-RHTEMP101A). Rainfall was also monitored daily from the INRAE CLIMATIK platform (https://intranet.inrae.fr/climatik/) managed by the AgroClim laboratory (Avignon, France). The herbage height was measured in each pasture using a grazing stick at 25 points along two (for SP) or four (for LP) transects per pasture on days 35, 50, and 73. Botanical composition, defined as the proportion of legumes or grass in eight (for SP pastures) or sixteen (for LP pastures) 0.25 m^2 plots, was also measured on days 35, 50, and 73. To estimate the plant biomass in the pastures, four (for SP) or eight (for LP) 0.25 m^2 plots were collected at a distance of 0.5, 2, 4, and 6 m from the exit hatch along two (for SP) or four (for LP) transects on days 35, 50, and 73 by cutting all the grass inside the plots to a height of 2 cm from the ground. The samples were pooled and placed in micro-perforated bags and stored at 4 °C until analysed using the methods described by the E.G.R.A.N (2001) group as most appropriate for rabbit nutritional experiments, i.e., DM (48 h at 60 °C; ISO 6496:1999 method), ash, CP (N × 6.25, Dumas method, ISO 16634:2004 method), and fibres (ADL, ADF, and lignin; Van Soest et al. (1991) method).

Spatial distribution of the rabbits

The spatial distribution of the rabbits in the pens and in the pastures was evaluated six times a week (i.e., three times a day, two days a week) for six weeks, i.e., on Mondays and Thursdays at 0730 h, 1400 h and 1800 h. We distinguished the genotype (i) in three 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, and (ii) in four areas in the pastures: (A1) from 0 to 2.5 m from the exit hatch, (A2) from 2.5 to 5.0 m, (A3) from 5.0 to 7.5 m, and (A4) from 7.5 to 10.0 m.

Behavioural evaluation

The behaviour of the animals was assessed for 10 minutes using the scan-sampling method, which consists of scanning the whole group of animals and recording each individual behaviour (Altmann, 1974), with direct observations simultaneously in the pens and on the pastures. Observations were made in the morning (between 0700 h and 0900 h just after sunrise), in the afternoon (between 1300 h and 1500 h), and in the evening (between 1800 h and 2000 h just before sunset) on rabbits aged 44, 58, and 70 days old. Two pairs of trained observers were required at each observation. The following behaviours described by Podberscek et al. (1991) were recorded: moving (hopping and walking), maintenance (grazing, eating, and resting), comfort (grooming), exploration and alertness (rearing, sniffing, stamping, and paw scraping), interaction (between two or more rabbits: side-by-side, allo-grooming, nose-to-nose, capering, and high-speed chase). The results are expressed as percentages of the total observations.

Hair corticosterone level

A patch of hair (5 × 5 cm) was shaved using small clippers from 80 rabbits (five males and five females of each genotype chosen from each of the four repetitions of each LP and SP outdoor grazing area) from each animal's back just behind the shoulder blades at 37 days of age, and the hair was then discarded. Then, at 72 days of age, approximately 250 mg of hair was collected by shaving following the same protocol. The samples were stored at −20 °C until the extraction of corticosterone. The method used was an adapted ELISA corticosterone immunoassay: we used a competitive immunoassay kit designed for the quantitative measurement of salivary cortisol (Salimetrics® Cortisol Enzyme Immunoassay kit) following the procedure developed by Salimetrics. Optical density was read on a Glomax spectrophotometer (Promega) at 450 nm and 490 nm. The concentration of each sample was determined by interpolation using a 4-parameter non-linear regression curve fit (Myassays software) and converted into pg/mg considering dilution factors.

Rabbit health

The health status of the animals was checked once a week between days 31 and 67 (the following criteria were annotated: healthy as no signs of disease, thinness, digestive problem, and abscess). Mortality was monitored daily. Coccidia oocysts were counted on 9 g of faeces taken once a week in each pen. Samples were collected on a steel shelf placed under one of the two platforms of each pen and were then stored at 5 °C for no more than 3 days before analysis according to the McMaster method (Gibbons et al., 2005).

Statistical analysis

All analyses were performed using R statistical software version 4.0.3 (R Core and Team, 2020). The herbage height data were analysed using independent linear models for each day of measurement. The biomass available in pastures on days 35, 50, and 73 were analysed using a Poisson regression model including pasture area (two levels: LP or SP), day (three levels: 35, 50, and 73 days), and their interaction as fixed effects. The live weights of the rabbits at 31 and 67 days of age and the average daily gain in this period were analysed with a mixed model including pasture area, genotype (two levels: 1001 and PS119), and their interaction as fixed effects and the animal as random effect. Mortality between 31 and 67 days of age was analysed as a binary trait (0 = dead and 1 = alive) using a logistic regression model with pasture area, genotype, and their interaction as fixed effects. The spatial distribution data was analysed using a Poisson regression model including pasture area (two levels: SP and LP), genotype (two levels: 1001 and PS119), and their interaction as fixed effects. The proportions corresponded to the total number of rabbits observed at each place over the total number of observations. The hair corticosterone level was analysed using a linear model including pasture area, genotype, sex, and their interactions as fixed effects. The Coccidia oocyst count was analysed using a Quasi-Poisson regression model including pasture area, rabbit age, and their interaction as fixed effects. Behavioural data were analysed with zero-inflated negative binomial regression including pasture area, genotype, and their interaction as fixed effects for each observation site (pastures or pens). All non-significant interactions were not described.

Results

Environmental conditions and herbage allowance

Average indoor and outdoor temperatures over the experiment were 17.6 °C (min 6.6 °C and max 27.1 °C) and 12.0 °C (min −1.9 °C and max 23.8 °C), respectively. Before allowing rabbits to access the pastures, the overall herbage height was 16.3 ± 0.5 cm for SP and 14.1 ± 0.3 for LP (P < 0.05); see Table 1. The botanical composition of the pastures was homogenous between SP and LP, as the proportion of grass ranged from 79 to 84%. The chemical composition of the meadow was 40.2% DM, 6.9% CP, 20.1% NDF, 10.0 ADF, 2.8% lignin, and 5.8% ash on a raw basis. Between rabbits aged 35 and 73 days, the proportion of grass and legumes decreased by 44.3% and by 73.3%, respectively, for SP pastures, and by 17.9 and 47.5%, respectively, for LP pastures (P < 0.05). The proportion of bare ground increased from 0.6% on day 35 to 47.5% on day 73 in SP pastures (P < 0.05) and from 0.3 to 7.8% in LP pastures (P < 0.05). In the same period, the proportion of stems increased from 0.0 to 2.5% in SP pastures (P = 0.12) and from 0.0 to 14.7% in LP pastures (P < 0.05). The mean amount of biomass available was similar between LP and SP pastures on day 35 (124.2 vs 159.9 g/m^2, respectively; P = 0.88; Fig. 1) and then decreased at a similar rate in the first two weeks of the trial (between day 35 and day 50) on LP and SP pastures (−70.2 vs −74.9%, respectively; P = 0.99). However, between day 50 and day 73, the amount of biomass available remained similar in LP pastures (from 36.9 to 36.2 g DM/m^2; P = 1.00) but decreased drastically in SP pastures (from 40.1 to 2.1 g DM/m^2; P = 0.12).

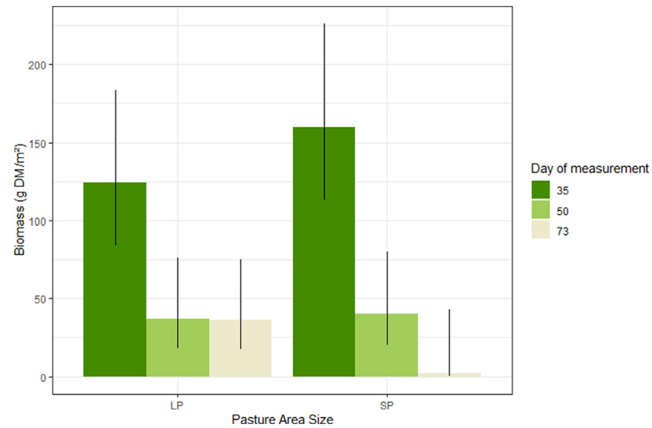

Fig. 1. Biomass available in the pastures (g DM/m^2) according to pasture size [small pasture (SP): 30 m^2 vs large pasture (LP): 60 m^2] and the day of the experiment (days 35, 50, or 73). Error bars represent the 95% confidence limits.

Animal traits

The overall mortality rate was 9.9% over the entire growing period and showed no effect of pasture size, genotype, or between pasture size × genotype interaction (Table 2). All the rabbits that died had digestive problems (diarrhoea). Note that 14.3% of the deaths occurred in the first week postweaning; i.e., before the rabbits had outdoor access. Peak mortality occurred when the rabbits were aged between 38 and 45 days (33.3%). All the live rabbits that reached 73 days of age were clinically healthy (no diarrhoea, no stomach swelling, no grown hollow cheeks, no lethargy, and no weight loss). The live weights of rabbits at weaning were similar regardless of the pasture size or genotype, at an average of 887 ± 92 g (Table 2 & Supplementary Fig. S2). At 67 days of age, the PS119 rabbits were 331 g heavier than the 1001 rabbits (2 444 vs 2 113 g, respectively; P < 0.05), with no difference between rabbits that had access to a 30 m^2 (SP) vs a 60 m^2 (LP) pasture (2 273 ± 218 g vs 2 270 ± 213 g, respectively; P = 0.87). Hair

Table 1
Means (±SE) for herbage height (cm) and percentage of grass, legumes, bare ground, and stems in the pastures according to pasture size [small pasture (SP): 30 m^2, or large pasture (LP): 60 m^2] and day of measurements (days 35, 50, or 73).

	Pasture Size			P-value
	SP	LP	RSD	
Herbage height (cm) on day[1]				
35	16.3	14.1	6.1	<0.05
50	5.9	9.9	5.1	<0.05
73	1.1	2.7	1.4	<0.05
Grass (%) on day[2]				
35	78.7 ± 3.2	83.7 ± 2.1	NE[3]	0.20
50	78.7 ± 3.2	90.3 ± 1.6	NE	<0.05
73	44.4 ± 3.9	69.1 ± 2.6	NE	<0.05
Legumes (%) on day				
35	20.6 ± 1.7	15.9 ± 2.1	NE	0.30
50	1.2 ± 0.1	1.5 ± 0.0	NE	1.00
73	5.6 ± 1.8	8.4 ± 1.6	NE	0.40
Bare ground (%) on day				
35	0.6 ± 0.0	0.3 ± 0.0	NE	1.00
50	9.4 ± 2.3	4.4 ± 1.1	NE	0.05
73	47.5 ± 3.9	7.8 ± 1.5	NE	<0.05
Stems (%) on day				
35	0.0 ± 0.0	0.0 ± 0.0	NE	1.00
50	10.6 ± 2.4	3.7 ± 1.5	NE	<0.05
73	2.5 ± 1.2	14.7 ± 1.9	NE	<0.05

1 Means for herbage height did not consider the percentage of bare ground (Day 35: 0 points for SP and LP; Day 50: 15 points for SP and 14 points for LP; Day 73: 76 points for SP and 25 points for LP).
2 Proportion of grass in relation to the number of measures for each group (40 or 80 measurement points for each SP or LP, respectively).
3 NE: not estimable.

Table 2
Rabbit growth and mortality according to pasture area [small pasture (SP): 30 m^2, or large pasture (LP): 60 m^2], and rabbit genotype (1001 or PS119).

	Pasture Size (S)		Genotype (G)			*P*-value[1]		
	SP	LP	1001	PS119	RSD	S	G	S × G
Number of rabbits at 31 days of age	96	96	96	96				
Liveweight (g) at 31 days of age	887	886	877	896	92.4	0.89	0.26	0.86
Number of rabbits at day 67	83	88	89	82				
Liveweight (g) at 67 days of age	2 273	2 270	2 113	2 444	157.4	0.87	<0.05	0.86
ADG[2] (g/d) between 31 and 67 days of age	39.9	39.7	35.3	44.3	3.7	0.91	<0.05	0.95
Mortality between 31 and 67 days of age (%)	12.6	7.4	7.2	12.9	NE[3]	0.17	0.14	0.57

[1] The interaction between pasture area and genotype was not significant ($P > 0.05$).
[2] ADG: average daily gain.
[3] NE: not estimable.

corticosterone build-up in the period (i.e., 37–73 days of age) was higher in 1001 rabbits than in PS119 rabbits (6.34 pg/mg vs 2.19 pg/mg of hair, respectively; $P < 0.05$; Fig. 2). Neither the size of the pasture nor the sex of the animals had an effect on this parameter. The number of oocysts per gram of faeces (**OPG**) changed during the experiment (Fig. 3). It started at 60 000 OPG at 36 days of age and then peaked at 46 days of age (75 725 OPG faeces) before decreasing by 85% between 46 and 67 days of age ($P < 0.05$). The size of the pasture had no effect on this parameter. The effect of genotype could not be studied as the faeces collected could not be individually identified.

Pelleted feed and hay intake

Over the entire growing period, the average feed intake was 110.7 g/day/rabbit and the average hay intake was 16.7 g/day/rabbit. There was a peak of feed intake at five weeks postweaning (average 141.8 g/day/rabbit) and a peak of hay intake at six weeks postweaning (average 37.6 g/day/rabbit), without differences between LP and SP (Fig. 4). Note however that a large amount of hay fell under the pens and was wasted, which means the consumption evaluation was biased and thus warrants being interpreted with a degree of caution.

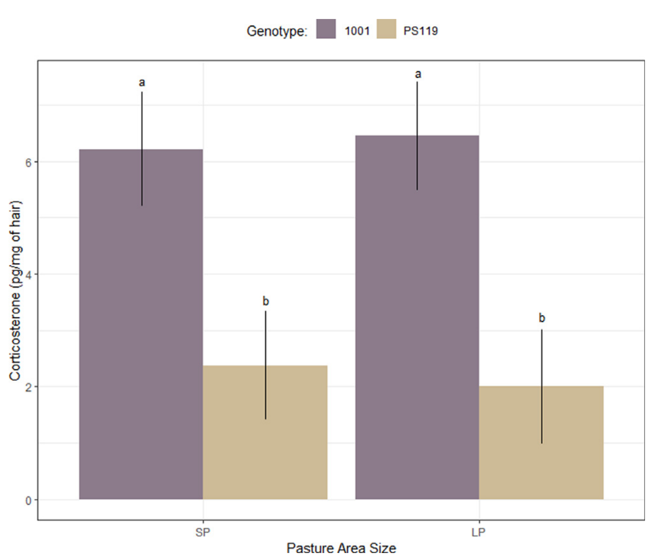

Fig. 2. Concentration of corticosterone (pg/mg of hair) in the rabbits' hair according to genotype (1001 vs PS119) and pasture size [small pasture (SP): 30 m^2 vs large pasture (LP): 60 m^2]. Error bars represent the 95% confidence limits. Superscripts with different letters (a,b) differ significantly at $P < 0.05$.

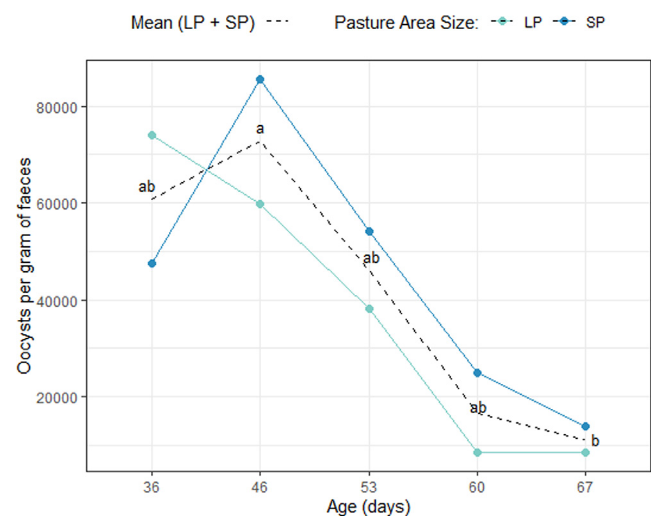

Fig. 3. Dynamics of oocyst counts (oocysts per gram of faeces; OPG) in rabbit faeces between 36 and 67 days of age according to pasture size [small pasture (SP): 30 m^2 vs large pasture (LP): 60 m^2]. Superscripts with different letters (a,b) differ significantly at $P < 0.05$.

Behavioural evaluation

Spatial distribution

Throughout the entire period, rabbits were more frequently observed outside in the pastures than indoors in the pens (53.9 vs 46.1%, respectively; $P < 0.05$; Table 3). When they were outside, the rabbits were observed more frequently in the A1 area than in the A2, A3, and A4 areas (35.9 vs 23.3, 15.6, and 25.2%, respectively; $P < 0.05$; data not shown). When they were inside, rabbits were observed more frequently on the floor below the platforms (80.9% were on the floor below the platform from the hatch side) than on the floor between the platforms or on top of the platforms (62.1 vs 30.6 vs 7.3%, respectively; $P < 0.05$). The SP-group rabbits were seen less frequently in the A1 area than the LP-group rabbits (14.0 vs 24.9%, respectively; $P < 0.05$). The time of day had an effect on the spatial distribution of the animals: the rabbits were more frequently observed in the pastures in the morning and in the early evening than in the afternoon (on average 75.1% and 70.9 vs 15.5%, respectively; $P < 0.05$; see Supplementary Fig. S3).

Expression of specific rabbit behaviours

Comparison of rabbit behaviour in pastures and in pens according to the size of the pasture area (Fig. 5A and B). Outside in pastures, regardless of age and the time of day, the behaviours *Walking* and *Grazing* were more frequent in the LP group than in the SP group (on average 5.7 vs 4.7% and 25.4 vs 21.5%, respectively; $P < 0.05$). *Rearing* behaviour was observed more frequently in the SP than in the LP group (on average 7.5 vs 5.8%, respectively;

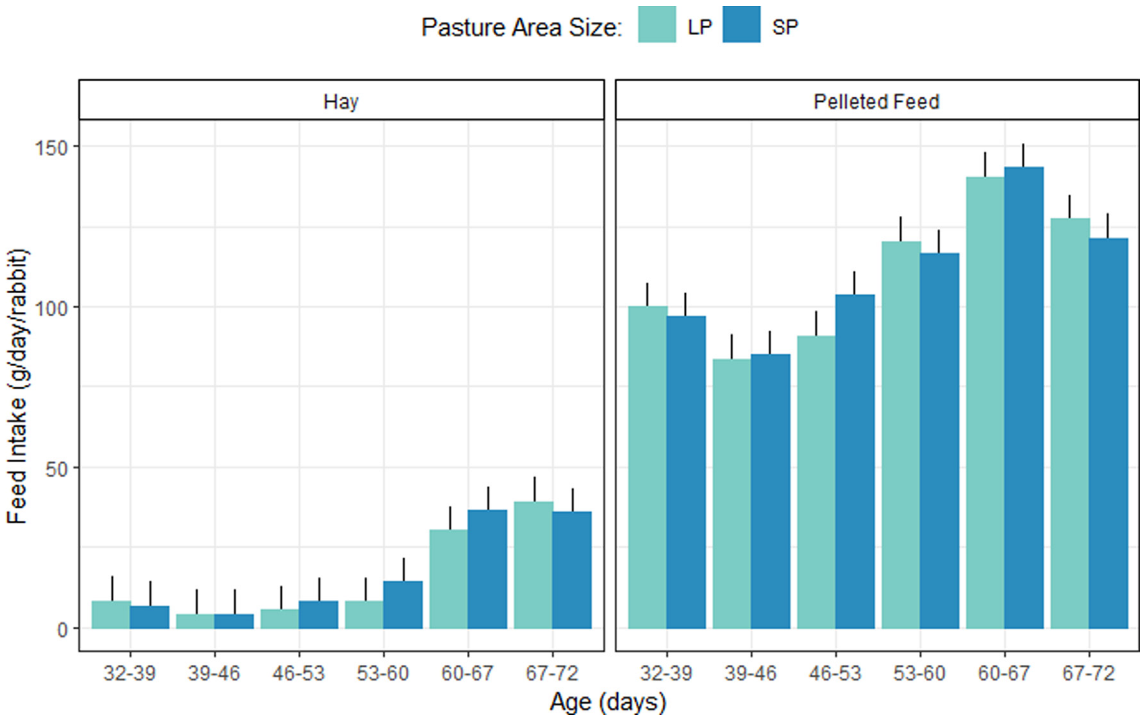

Fig. 4. Hay and pelleted feed intake consumed by rabbits (g/day/rabbit) according to pasture area [small pasture (SP): 30 m^2 vs large pasture (LP): 60 m^2] and the age of rabbits (from 32 to 72 days of age). Error bars represent the 95% confidence limits.

Table 3
Mean (±SE) spatial distribution of rabbits (%; expressed as frequency of total observations) according to pasture size [small pasture (SP): 30 m^2, or large pasture (LP): 60 m^2], and rabbit genotype (1001 or PS119) between 42 and 67 days of age.

	Pasture Size (S)		Genotype (G)		*P*-value		
	LP	SP	1001	PS119	S	G	S × G
Distribution of rabbits							
Inside (pens)	45.3 ± 4.9	46.8 ± 4.9	45.0 ± 4.8	47.1 ± 4.9	0.82	0.76	0.92
Outside (pastures)	54.7 ± 5.3	53.2 ± 5.3	55.0 ± 5.3	52.9 ± 5.2	0.84	0.78	0.92
Zones inside the building							
Between platforms	12.4 ± 2.6	15.7 ± 2.9	13.3 ± 2.7	14.7 ± 2.8	0.39	0.74	0.85
Below platforms	30.0 ± 3.9	27.0 ± 3.6	28.9 ± 3.8	28.0 ± 3.7	0.58	0.87	0.95
Above platforms	2.7 ± 1.2	3.9 ± 1.5	2.6 ± 1.2	4.2 ± 1.5	0.49	0.35	0.74
Zones outside							
[0; 2.5[m[1]	24.9 ± 3.6	14.0 ± 2.7	19.3 ± 3.2	18.1 ± 3.2	<0.05	0.94	0.51
[2.5; 5[m	9.3 ± 2.2	15.8 ± 2.9	13.1 ± 2.6	11.2 ± 2.5	0.08	0.68	0.63
[5; 7.5[m	6.5 ± 1.8	10.3 ± 2.3	8.2 ± 2.0	8.1 ± 2.1	0.19	0.93	0.84
[7.5; 10] m	13.8 ± 2.7	13.0 ± 2.6	13.4 ± 2.6	13.4 ± 2.6	0.81	0.99	0.72

[1] Distance from the hatch: [0; 2.5[for A1; [2.5; 5[for A2; [5; 7.5[for A3; [7.5; 10] for A4.

$P < 0.05$). In the pens, the behaviours *Eating* and *Rearing* were more frequent in the SP group than in the LP group (on average 22.0 vs 18.7% and 5.2 vs 4.5%, respectively; $P < 0.05$).

Comparison of rabbit behaviour on pastures and in the pens according to genotype (Fig. 5C and D). Outside in pastures, regardless of age and time of day, *Hopping*, *Resting,* and *Side-to-Side* were more frequent in PS119 rabbits than in 1001 rabbits (11.0 vs 9.1%, 27.0 vs 23.7%, and 10.7 vs 7.8%, respectively; $P < 0.05$), whereas *Sniffing* was more frequent in 1001 rabbits than PS119 rabbits (7.8 vs 4.8%, respectively; $P < 0.05$). In the pens, *Resting* and *Sniffing* were more frequent in 1001 rabbits than in PS119 rabbits (32.4 vs 30.0% and 4.0 vs 3.1%, respectively; $P < 0.05$).

Author's points of view

The aim of this study was to evaluate the effects of pasture size and rabbit genotype on growth, health, behaviour, and corticosterone levels in fattening rabbits. The results show that (i) the size of the outdoor grazing area influenced the grass availability and thus rabbit behaviour: *Walking* and *Grazing* behaviours were observed more frequently in larger than in smaller pastures, (ii) the rabbit genotype influenced growth traits and rabbit behaviour: PS119 rabbits were heavier at the end of the fattening period, had lower hair corticosterone levels, and *Hopping*, *Resting,* and *Side-to-Side* were observed more frequently in the grazing area.

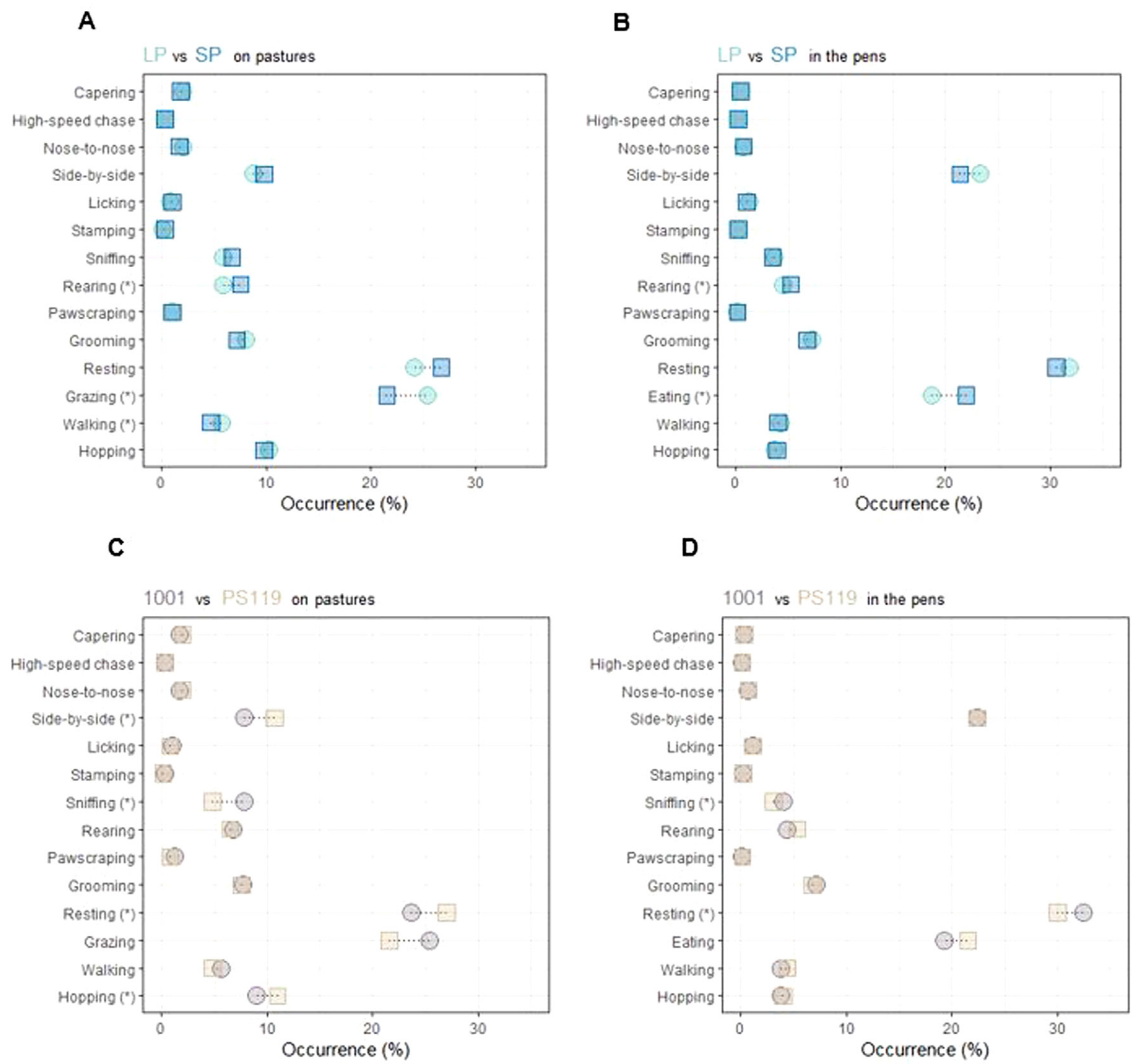

Fig. 5. Effect of pasture size [small pasture (SP): 30 m^2 vs large pasture (LP): 60 m^2] and genotype (1001 or PS119) on occurrences of behaviours in rabbits (%) measured at 44, 58, and 70 days of age; aggregated data. **A** and **C:** in pastures or **B** and **D**: in pens. The (*) marks mean that the differences are statistically significant ($P < 0.05$).

Pelleted feed and hay consumption, health, and general behaviour of rabbits in a system with outdoor access

During the entire growing period, the pelleted feed intake was similar between rabbits in small pastures (111.0 g/day/rabbit) and large pastures (110.3 g/day/rabbit). The nutritional contribution of grazing, although weak (199.9 and 310.4 g DM/rabbit in SP and LP, respectively, over the entire growing period), may have lowered pelleted feed consumption in the periods where forage biomass was most abundant. Hay intake during the entire growing period was low (approximately 17 g/day/rabbit) compared to the value reported by Goby et al. (2013; 84 g/day/rabbit). Although the hay consumption data warrant being interpreted with a degree of caution, it confirms the preference of the rabbits for green and tender plants rather than dry ones (Gidenne et al., 2015). Prebble et al. (2015) reported a lower hay intake when the front paws are placed on the hayrack than in a natural grazing posture from either the floor or while sitting in the hayrack. Here, most of the rabbits ate the hay with the front paws placed on the hayrack (see Supplementary Fig. S4), although we also frequently observed that rabbits sat in the hayrack (see Supplementary Fig. S5). Moreover, the space between the bars of the hayracks was too wide to catch and hold the hay, which facilitated hay waste. It would be preferable to lower the hayracks to the height of the rabbits' mouths so that they do not have to rear up to eat, and to narrow the bars to reduce hay waste.

Coccidiosis, due to infection with one or several species of the genus *Eimeria*, is very common in domesticated rabbits (Pakandl, 2009). Rabbits are orally infected by consuming feed or water contaminated with oocysts. Pasture-raised rabbits are particularly exposed to this disease (Legendre et al., 2021) as the wild fauna plays a key role as a reservoir (Tizzani et al., 2011). This makes it useful to evaluate contamination by counting oocysts, even if the presence of oocysts in faeces does not necessarily equate with the onset of a clinical form (Pilarczyk et al., 2020). Here, the number of OPG in faeces was high before the rabbits had outdoor access, and it reached a peak one week after the animals went outside.

In the pens, the rabbits preferred to be on the floor below the platforms. We assume that this area mimics a shelter for rabbits (Lombardi et al., 2007; Beja et al., 2007), as wild rabbits tend to remain in covered areas and burrows during the daytime (Kolb, 1986). The time of day strongly influenced the spatial distribution of rabbits, as they were more frequently observed in the pastures in the morning and in the evening (see Fig. 3). This agrees with Mykytowycz and Rowley (1958), who showed that wild rabbits were mostly found outside their burrows from 1600 to 0600 h.

Resting was the most frequently expressed behaviour regardless of the location of the observation, followed by *Grazing* in pasture areas and *Eating* in pens. This is consistent with Prebble et al. (2015), who showed that rabbits spend 30–70% of their time grazing but remain mainly inactive in daylight. Behaviours linked with vigilance (*Rearing* and *Stamping*) were relatively rare. Monclús and Rödel (2009) reported that young animals exhibited lower vigilance rates because vigilance competes with energy gain through feed intake.

Effect of genotype

The PS119 semen used to produce the crossbreed rabbits is marketed as producing robust animals, and the PS119 rabbits had higher live weights and higher average daily gains than the 1001 rabbits produced with 1001 semen. Thus, the paternal line is an interesting lever for choosing the most suitable genetic type for breeding rabbits in a system with outdoor access. Fetiveau et al. (2021) showed that providing outdoor access to purebred 1777 rabbits slightly reduced their growth compared to purebred 1777 rabbits with no outdoor access. In our case, an increase in pasture size associated with a potential increase in physical activity did not appear to have an impact on the growth of both of the crossbred genotypes used. These observations may be related to the use of crossbred rabbits. Indeed, Jaouzi et al. (2004) showed that crossed-breed rabbits are less impacted in terms of growth traits than purebred ones. The pelleted feeding strategy chosen in this trial may also have played a role in this result. Indeed, Fetiveau et al. (2021) used a restricted feeding strategy (85 g/kg of live weight + 15 g/rabbit every 7 days), while in this present trial, the rabbits were fed *ad libitum*.

On pastures, PS119 rabbits more frequently expressed the *Resting* behaviour than did 1001 rabbits. Conversely, 1001 rabbits more frequently expressed the *Sniffing* behaviour, both in pastures and in the pens. These observations indicate a more relaxed behavioural pattern in PS119 rabbits, as further suggested by the lower corticosterone level in their hair, which suggests that they may be less affected by their environment. Corticosterone levels are known to be age-dependent, with higher levels in young animals compared to in adults (Heimbürge et al., 2019), and also genotype-dependant (Redgate et al., 1981). In future trials, it would be informative to compare corticosterone levels in the hair of rabbits of similar age and genotype reared in cages vs free-range in order to evaluate the consequence of outdoor access on corticosterone levels. Indeed, D'Agata et al. (2009) showed that fattening rabbits kept in an outdoor system had lower fear levels in the open field test compared to indoor rabbits, and they argued that outdoor-reared animals were probably exposed to a greater number of stimuli that lead them to be less emotionally reactive and better adapted to face stressors.

Effect of pasture size

Before rabbits were given outdoor access, a difference in herbage height without a difference in the biomass production can be explained by great variability in herbage density. After rabbits were allowed access to outdoor grass, the available herbage in the 30 m^2 pastures was entirely consumed in 20 days, leaving only small and dry patches of short grass on otherwise bare ground. In the 60 m^2 pastures, grassy biomass, mainly short grass, remained available for grazing right up until the end of the fattening period. Here, we found that providing a 60 m^2 pasture with *ad libitum* pelleted feed and hay to 24 rabbits appeared to be sufficient in the season studied to enable rabbits to express grazing until the end of the fattening period.

The larger space available and higher biomass provided by LP pastures resulted in more observations of *Walking* and *Grazing* by rabbits in the pastures. In return, the smaller space available and lower biomass offered in SP pastures resulted in more rabbits observed *Eating* in the pens. However, we cannot relate this observation to higher pellet feed and hay intakes, even at the end of the fattening period when there was no more grass on the smaller pastures, as our system could not reliably discriminate eating and chewing behaviours.

To conclude, this study showed that the size of the pasture area had no specific effect on rabbit growth or health. However, in the spring season, providing rabbits with a larger pasture area enhanced the expression of grazing behaviour, as there was still herbage present up until the end of the fattening period. It would be interesting to confirm these results in another season when the grass biomass could be less abundant. The rabbit genotype had an influence on their behaviour patterns, and animals from the line selected for robustness and growth traits appeared to cope better with the external stimuli, as suggested by lower corticosterone levels in their hair. The ability of all the animals to express their specific behaviours during the entire growing period together with the low incidence of digestive disorders in the present work is indicative of enhanced animal welfare. Further studies could use enrichment of the pastures to homogenise pasture use. Moreover, capturing the time budget of specific behaviours for each rabbit could help better characterise individual variability in behaviours in such a farming system.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.anopes.2023.100038.

Ethics approval

The animals were handled in accordance with the recommendations of the European Union (2010) and French legislation on the protection of animals used for scientific purposes (EU Directive 2010/63/EU, Official Journal of the French Republic (Decree No. 2013-118)). All the protocols were approved by the Ethics Committee no. 115 of the Ministry of National Education, Higher Education and Research (approval number 16330-2018072716211212).

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Declaration of interest

None.

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