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Ph.D. Course in
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Performance and Welfare of Reproducing Does and Growing Rabbits in Alternative Housing Systems

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Index

RIASSUNTO

Il comportamento dei conigli domestici si basa sul modello dei conigli selvatici che vivono in colonie e comprende, fra le altre, attività locomotorie ed esplorative. I conigli si spostano principalmente saltando, vivono in gruppo, stabiliscono gerarchie specifiche entro sesso e mostrano comportamenti aggressivi principalmente durante la formazione delle gerarchie, la stagione riproduttiva e in caso di competizione per le risorse disponibili. Nell'allevamento convenzionale, i conigli sono tenuti in gabbie individuali che limitano i loro comportamenti naturali e portano a comportamenti anomali. Sistemi alternativi come i recinti collettivi offrono più spazio e ampia possibilità per l'utilizzo di arricchimenti ambientali, migliorando il benessere dei conigli. Tuttavia, l'allevamento di conigli in gruppo presenta sfide riguardo l'aggressività e la gestione dello stato sanitario degli animali.

Il contesto è quello di una società che presta sempre più attenzione al benessere degli animali allevati, spingendo verso sistemi di allevamento più rispettosi degli animali. In particolare, c'è una crescente pressione da parte dei consumatori e degli attivisti per i diritti degli animali affinché si presti maggiore attenzione al benessere dei conigli da allevamento. Nonostante siano stati condotti diversi studi per migliorare il loro benessere e la loro salute, rimangono molte domande senza risposta, soprattutto riguardo il tipo di stabulazione.

In questa tesi sono stati considerati vari aspetti riguardanti l'allevamento in sistemi senza gabbie (cage-free), valutando l'uso di arricchimenti per migliorare il benessere dei conigli in accrescimento (Contributo 1), analizzando il benessere in sistemi di stabulazione diversi (Contributo 2), valutando l'effetto delle dimensioni del gruppo e del momento di formazione dei gruppi sul comportamento delle coniglie fattrici in attività riproduttiva e caratterizzando l'attività locomotoria, in particolare il salto, di questi animali in diversi momenti per avere maggiori informazioni sui loro fabbisogni (Contributo 3).

Nel primo Contributo della presente tesi, sono stati valutati gli effetti della disponibilità di blocchi di fieno da rosicchiare e della composizione del gruppo sulle prestazioni, il comportamento e la reattività di 288 conigli in crescita allevati in 18 recinti (16 conigli/recinto) dall'età di 31 a 73 giorni. La presenza dei blocchi da rosicchiare dei parchi ha influenzato marginalmente le prestazioni e l'etogramma, ma ha ridotto il tempo trascorso nell'area di riposo ($p<0,001$); ha aumentato il tempo trascorso in movimento durante il test di open field ($p<0,05$) e la percentuale di conigli che si sono avvicinati all'oggetto nel test oggetto ($p<0,05$). Per quanto riguarda la composizione del

gruppo in base al sesso, l’indice di conversione alimentare è stato più basso nei recinti con sole femmine e con femmine e maschi rispetto ai recinti con soli maschi ($p<0,05$). Durante il test di open field, i conigli dei recinti a sessi misti (sia maschi che femmine) hanno trascorso più tempo in movimento ($p<0,05$), mentre i conigli dei recinti con soli maschi hanno dedicato più tempo al self-grooming ($p<0,01$). I risultati relativi alla produzione, al comportamento e alla reattività indicano che la fornitura di blocchi da rosicchiare migliora il benessere, ma non giustifica il passaggio dall'attuale allevamento a sessi misti ad un allevamento a singolo sesso.

Nel secondo Contributo di questa tesi, sono stati confrontati la salute e il benessere dei conigli tenuti in quattro diversi sistemi di stabulazione (BI, gabbie bicellulari per conigli in crescita e gabbie standard per le femmine riproduttrici; DP, gabbie polifunzionali; gabbie arricchite; recinti collettivi) utilizzando misure basate su risorse, gestione e animali in condizioni di campo. Complessivamente, sono state visitate 12 aziende commerciali (3 aziende per sistema di stabulazione) durante tre stagioni (estate, autunno, inverno) in due occasioni: una visita pre-svezzamento per registrare informazioni sulle femmine riproduttrici e le nidiate; una visita pre-macellazione per registrare informazioni sui conigli in accrescimento. Durante la visita pre-svezzamento, le femmine riproduttrici degli allevamenti con gabbie standard hanno mostrato il peso vivo più basso (4431 g rispetto a 4765 g, 4914 g e 4968 g; $p<0,001$) e le peggiori condizioni corporee (punteggio: 1,91 rispetto a 1,94, 2,00 e 2,09; $p<0,001$) rispetto alle femmine degli allevamenti con gabbie DP, gabbie arricchite o con recinti collettivi. Il numero di coniglietti per nidiata più basso è stato riscontrato negli allevamenti che utilizzavano le gabbie standard e le gabbie DP rispetto a quelli con le gabbie arricchite o i recinti (8,08 e 8,21 rispetto a 8,61 e 9,18; $p<0,001$), mentre il peso dei coniglietti più basso è stato registrato per le nidiate degli allevamenti con gabbie standard e nelle gabbie arricchite. La prevalenza di problemi di salute non differiva tra le femmine o le relative nidiate tenute in diversi sistemi di stabulazione. Durante la visita pre-macellazione, il peso vivo dei conigli in accrescimento è diminuito da quelli degli allevamenti con gabbie arricchite a quelli con gabbie DP, recinti collettivi e gabbie bicellulari (2584 g rispetto a 2509 g, 2464 g e 2456 g; $p<0,001$). Per quanto riguarda i problemi di salute, è stata riscontrata una maggiore incidenza di dermatomicosi negli allevamenti con recinti e gabbie DP. Nel complesso, i risultati del presente studio non hanno evidenziato differenze significative di benessere animale e stato di salute delle femmine riproduttrici e delle loro nidiate o dei conigli in crescita tenuti in diversi sistemi di stabulazione sulla base degli indicatori inclusi nel protocollo utilizzato.

Nel terzo Contributo è stata caratterizzata l'attività motoria di coniglie fattrici allevate in colonia. Utilizzando 72 femmine riproduttrici alloggiate in altrettanti moduli individuali (53 cm x 92 cm; 0,5 m^2) che sono stati successivamente uniti per formare recinti collettivi con gruppi di due (N2) o quattro (N4). Il raggruppamento delle femmine è stato effettuato 12 giorni (T12), 15 giorni (T15) o 18 giorni (T18) dopo il parto. Quando i recinti collettivi sono stati formati, sono stati arricchiti di una piattaforma e un tubo nel caso dei parchi N2 e due piattaforme e due tubi nel caso dei parchi N4.

Il comportamento è stato registrato tramite videocamera nelle 24 ore successive sia al momento del raggruppamento sia a 3 giorni dopo. Le registrazioni video sono state utilizzate per misurare il numero di salti nelle diverse aree del parco, ovvero pavimento, piattaforma e tra piattaforma e pavimento. I dati sono stati raccolti per ciascuna femmina per 30 minuti nelle 24 ore di registrazione video, per un totale di 12 ore per femmina per parco per giorno. I salti sono stati identificati come singoli, doppi e tripli consecutivi. Quando erano più di tre, è stato specificato se i salti consecutivi erano diretti o non diretti (multidirezionali). Spostamenti eseguiti senza salti completi e quelli associati all'aggressione (fuga o combattimento) non sono stati considerati.

Le femmine riproduttrici hanno mostrato comportamenti di salto diversi quando sono state allevate in gruppi di diverse dimensioni e sotto diverse condizioni di gestione. Le osservazioni hanno rivelato che i salti singoli sono stati i più comuni, seguiti dai salti doppi e tripli. Tuttavia, i salti multipli (più di tre salti consecutivi) sono stati occasionali. Resta da verificare se questo risultato sia da attribuire alla vicinanza delle risorse come il cibo e l'acqua, alla disposizione specifica dei recinti con relativi arricchimenti o alla natura dei conigli stessi.

In conclusione, questa tesi contribuisce alle conoscenze sul benessere e sulla salute dei conigli in sistemi convenzionali e sistemi cage-free. Resta un urgente bisogno di ulteriori ricerche volte a perfezionare le strategie di stabulazione e gestione, garantendo la loro efficacia nel promuovere il benessere degli animali e determinandone la praticità su scala più ampia.

ABSTRACT

The behavior of domestic rabbits is based on the model of wild rabbits living in colonies and includes locomotor and exploratory activities. Rabbits move primarily by hopping, they live in groups, form sex-specific dominance hierarchies, and display aggressive behaviors primarily during hierarchy formation, the breeding season, and in case of competition for resources. In conventional breeding, rabbits are kept in individual wire cages, which limits their natural behaviors and leads to abnormal behavior. Alternative collective systems such as parks offer more space and ample opportunity for the use of environmental enrichments, improving the welfare of rabbits. However, group rearing of rabbits poses serious challenges regarding aggression among animals and their health.

These facts fit into a context in which society pays more and more attention to the welfare of farmed animals, pushing towards more animal-friendly farming systems. There is growing pressure from consumers and animal rights activists to pay more attention to the welfare of farmed rabbits. Although several studies have been conducted to improve their welfare and health, many unanswered questions remain, especially regarding the types of housing that can contribute to good health and appropriate behaviors.

In this thesis various aspects regarding breeding in cage-free environments have been explored, such as environmental enrichment to improve the welfare of growing rabbits (contribution 1), analyzing welfare in different housing systems (contribution 2), evaluating the effect of group size and grouping time on the behavior of the reproductive rabbits and the characterization of the locomotory behavior and requirements, with special emphasis on hopping (Contribution 3).

As for the first Contribution, the effects of the provision of hay blocks to gnaw and the group sex-composition (females, males, mixed) were evaluated on the performance, behavior, and reactivity of 288 growing rabbits raised in 18 parks (16 rabbits/park) from 31 to 73 days of age. The presence of gnawing blocks marginally affected performance and budget time, but reduced time spent in the rest area compared to parks without blocks ($p<0.001$); increased the time spent moving during the open field test ($p<0.05$) and the percentage of rabbits that approached the object in the novel object test ($p<0.05$). Regarding group composition by sex, the feed conversion ratio was lower in parks with only females and mixed sexes compared to parks with only males ($p<0.05$). During the open field test, rabbits in parks with mixed sex spent more time moving ($p<0.05$), while in parks with

only males they groomed themselves for a longer period (p<0.01). The production, behavioral and responsiveness results indicate that the provision of gnawing blocks improves welfare but does not support the transition from current mixed to single-sex farming.

In the second contribution, the health and welfare of rabbits kept in four different housing systems were compared (BI, two-cell cages for growing rabbits and standard cages for breeding females; DP, dual-use cages; enriched cages; parks) using measures based on resources, management and animals through a protocol carried out directly on the farm. Overall, 12 commercial farms (3 farms per housing system) were visited during three seasons (summer, autumn, winter) on two occasions: a pre-weaning visit to record information on breeding females and litters and a pre-slaughter visit to record information about growing rabbits. During the pre-weaning visit, reproducing does in standard cages showed the lowest live weight (4431 g vs. 4765 g, 4914 g, and 4968 g; p<0.001) and worst body condition (1.91 vs. 1. 94, 2.00 and 2.09; p<0.001) compared to females in DP cages, enriched cages, and parks. The lowest number of litters was found in standard cages and DP cages compared to enriched and park cages (8.08 and 8.21 compared to 8.61 and 9.18; p<0.001), whereas the weight of the lowest litter size was recorded for pups in standard cages and enriched cages. The prevalence of health problems did not differ between females or their litters kept in different housing systems. At the pre-slaughter visit, the live weight of the growing rabbits decreased from those in enriched cages to those in DP cages, parks, and BI cages (2584 g versus 2509 g, 2464 g, and 2456 g; p<0.001). Regarding health problems, a higher incidence of dermatomycosis was found in DP parks and cages. Overall, the results of the present study showed no significant differences in the welfare and health of reproducing does and their litters or growing rabbits kept in different housing systems based on the indicators included in the tested protocol.

In the third contribution, the hopping pattern of rabbit does raised in colonies was analyzed. Using 72 breeding female rabbits housed in as many individual modules (53 cm x 92 cm; 0.5 m^2) which were subsequently combined to form parks for the collective housing of breeding females in groups of two (N2) or four (N4). Aggregation of females was performed 12 days (T12), 15 days (T15) or 18 days (T18) after parturition. When the collective parks were formed, they were enriched with one platform and one tube in the case of the N2 parks and two platforms and two tubes in the case of the N4 parks.

Behavior was recorded via video camera over the next 24 hours both at the time of aggregation and 3 days after aggregation. Video recordings were used to measure the number of hops in different areas of the park, i.e., floor, platform and between platform and floor. Data was collected for each female for 30 minutes over the 24 hours of video recording, for a total of 12 hours per female per park per day. The hops were identified as consecutive singles, doubles and triples. When there were more than three, it is specified whether the consecutive hops were directed or undirected (multidirectional). Moves performed without full jumps and those associated with aggression (flight or fight) were not considered.

Breeding females exhibited different hopping behaviors when reared in different group sizes and under different management conditions. Observations revealed that single hops were the most common, followed by double and triple hops. However, multiple hops (more than three consecutive hops) were rare. It is yet to be determined whether this rarity is due to the proximity of resources such as food and water, the specific layout of the enclosures with related enrichments, or the intrinsic nature of the rabbits themselves.

In conclusion, this thesis contributes to the existing body of knowledge on the welfare and health of rabbits in standard cage and in cage-free systems. However, it also highlights the existence of numerous unanswered questions in this area and the need for further research aimed at refining housing and management strategies, ensuring their effectiveness in promoting animal welfare and determining their practicality on a larger scale.

1. INTRODUCTION

1.1. Domestic Rabbit

Rabbit (*Oryctolagus cuniculus*) it's a widespread species in every continent of the world, except for Antarctica; it is present both in wild and domestic form (López et al., 2020), with few differences between the two types. It belongs to the order of Lagomorpha and the family of Leporidae jointly, for example, hare, cottontail rabbit and pygmy rabbit, and, unlike rodents, lagomorphs possess two pairs of incisors in the upper dental arch (one of them is behind the other one), against the single pair of rodents (Gamberini, 2009).

This species has a triple attitude as lab, pet and meat animals, besides hair and fur production. Rabbit for fur, however, is often considered a byproduct because rabbits are bred for meat and European techniques for meat production are incompatible with quality standards of fur pelts (Lebas et al., 1997). The success of this type of production in Italy is due the possibility to rear these animals in intensive form and because of fast growth and reproductive rate, which allow shorter production cycles compared with many animals (Gamberini, 1993, 2009).

As for meat production, the leading country is China, followed by North Korea and EU-27 (FAO, 2021). In 2018, world production was around 901,477 tons, 465,733 tons of which were produced by China (mainland), 150,705 tons by North Korea, and 140,182 tons by EU-27 (Figure 1). In general, the production in last years has grown until 2014 (peak of 1,355,617 tons) and then decreased, like shown in Figure 2 (FAOSTAT, 2021). However, FAO data are to be handled carefully because they are deemed unreliable, as cited by Nature Plants (2019), so these will be a simple reference.

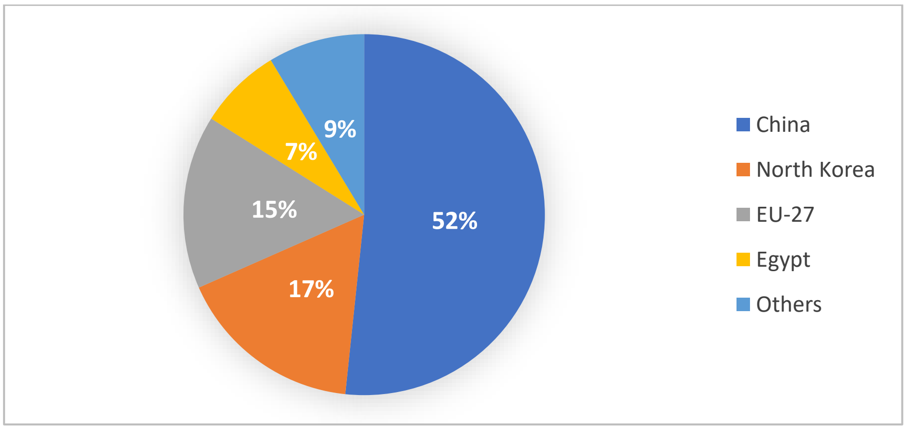

Figure 1. *Distribution of worldwide rabbit meat production in quantitative terms, expressed as a percentage (FAOSTAT, 2021).*

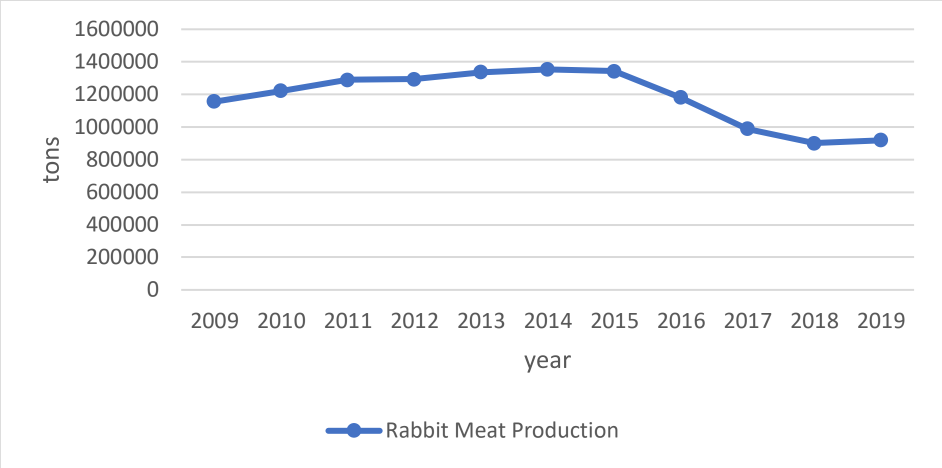

Figure 2. *Rabbit Meat Production in the World in years 2009-2019 (FAOSTAT, 2021).*

The decline in rabbit meat production, that can be observed in Figure 2, can be attributed to several reasons, among which we can identify a growing perception of rabbit as a pet rather than a meat animal (Petracci et al., 2018). So, rabbit production for meat is driven by a smaller number of countries than the production of other meats such as beef, pork, and poultry (Gamberini, 1993); the decline is attributable also to less competitive market prices compared to other types of meat (Petracci et al., 2018).

In the European Union, producing the 18.8% of rabbit meat world production in 2018, Italy is the third producer after Spain (55,824 tons, 39.82% of EU production) and France (43,886 tons, 31.29% of EU production), with 23,741 tons (16.93% of EU) (FAOSTAT, 2021). In terms of number of farmed animals, the production in UE was the sixth in 2017 (after that of the other farmed species), while in terms of volume of meat was the eighth (EU Commission, 2017). The slaughtering live weight of rabbits in Italy is between 2.2 and 3.0 kg at 65-90 days of life, whereas it is 2.0-2.2 kg in Spain and 2.3-2.4 kg in France (Luzi et al., 2009; EFSA, 2020). Italian production is almost self-sufficient, therefore imports, which remain intra-EU, are low (Gamberini, 2009; Trocino et al., 2019b).

Currently, in Italy, as can be seen in Figure 3, rabbit production follows a trend similar to the world one: it grew until 2016 and then suffered a decline (FAOSTAT, 2020; ISTAT, 2020), for reasons, also attributable in this case to the issue of the rabbit recognized by consumers as a pet and with higher production costs respect other meat types such as pork and poultry. Veneto Agricoltura in 2009 calculated the production costs of rabbit meat per kg was € 1.80/kg: 57.11% of costs from feeding, 18.86% from labor, 13.64% from drugs, and 10.39% from other stuffs; more recently Mondin et al., 2021 calculated the production cost seems to have dropped to €1.58/kg: 66.7% of costs from feeding, 4.6% from drugs, 3.4% from electricity, 7.4% from reproducing, 13.1% shed costs, 5.3% cages costs; the costs were higher than, for example, swine meat production (€ 1.30/kg) and poultry meat production (€ 1.04/kg) (C.R.P.A., 2011a, b). Most of the rabbit farms, about 75%, are in the North of Italy, especially in the North-East (about 55%) (ISTAT, 2013).

Rabbit meat consumption in EU is 0.1-0.5 kg per person per year in Belgium and Germany, 0.5-1 kg in France and Italy, 1-1.5 kg in Spain and Portugal, and with more than 3 kg in Malta (EU Commission, 2017).

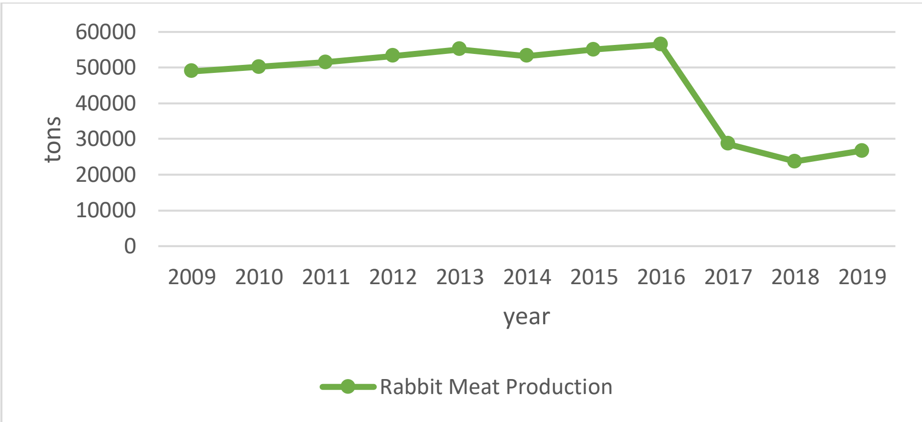

Figure 3. *Rabbit Meat Production in Italy in years 2009-2019 (FAOSTAT, 2020).*

1.2. Rabbit Ethology

Since the domestication of the rabbit was rather recent compared to other farmed species, the behavioral repertoire has remained practically unchanged. Thus, the wild rabbit is used as a reference for the ethology of the domestic one (Luzi et al., 2009).

In details, the European wild rabbit is a gregarious animal that lives in colonies inside burrow systems (which owe the name "*cuniculus*" of the species) (Cowan, 1987), generally composed by 2-9 females and their offspring, and 2-3 males, and characterized by a linear hierarchy for each sex (Bell, 1983; Surridge et al., 1999). Rabbits have a behavioral repertoire that can be divided into the following behavioral categories: locomotor and exploratory activities, nutrition, "self-care" and rest activities, social, aggressive and reproductive behaviours, maternal behaviours, which are detailed hereby.

Locomotor and Exploratory activities. A peculiarity of this species is the movement characterized mainly by hops, where a single hop is composed of a push of the hind legs and a landing on the front ones that allow to keep the body balanced. With a single hop rabbits can reach great distances and eventually great heights, and they can also do zigzag movements. All these characteristics allow these animals to escape from predators. As for exoloration and involved senses, rabbits explore the environment outside the burrows using hearing, sight, smell and taste, through standing on the hind legs, sniffing, licking or gnawing (e.g., wood) (Trocino and Xiccato, 2006; IGN, 2011).

Nutrition behaviours. Rabbits, to avoid predators, go out of their burrows during twilight-night hours to find feedd and water, whereas when rabbits are inside burrows, during night-dawn hours (Luzi et al., 2009), they practice caecotrophy, which consists in the fermentation of undigested nutrients in the cecum by microorganisms. Fermentation produces volatile fatty acids, absorbed in the intestine itself by epithelium, and amino acids, vitamins, and minerals. Then, the so-called "soft faeces" or "caecotrophs" are produced by rabbits, ingested by the animal directly from the anus. Digestion, therefore, continues with a further absorption of nutrients, while the rest is soon expelled through the so-called "hard faeces". The formation of hard faeces occurs a few hours after ingestion of the feedd, therefore under farming conditions during the afternoon-night period, whereas that of soft faeces occurs mainly during the daytime (Xiccato and Trocino, 2008). Grass and vegetables, in addition to providing nourishment to rabbits, allow them to file their teeth (which are constantly growing), thanks to the silica crystals, preventing overgrowth problems. According to EFSA (2005, 2020), wild rabbits spend up to 60% of the day in nutrition behaviours, based on the availability of feed.

Comfort activities and Rest. In the wild, rabbits spend the daytime inside the tunnels in activities such as: self-grooming (licking their own head or body, or caresses the head with their front legs, especially during night-dawn time; 12-20% of the day; Luzi et al., 2009).

Social, Aggressive and Reproductive behaviours. Rabbits, as mentioned, are gregarious animals that live in groups inside warrens (the burrow systems). The dominance hierarchy is sex-specific; indeed, the dominant male submits only the other males (and has the priority to search females for mating and search the best places to eat), and the dominant female submits only the females (DiVincenti and Rehrig, 2016; Graf et al., 2011; Munari et al., 2020). In nature, usually fights and aggressions occur only during the establishment of the hierarchy, the reproductive season between November and June (Gonçalves et al., 2002), and because of competition over nests and for territory (Graf et al., 2011; Gerencsér et al., 2019). During breeding periods, when social activities are at a maximum (high social pressure), home ranges are greater than in other months. Number of aggressions between males or females are higher in larger populations, both against foreign rabbits and fellow rabbits (Myers and Poole, 1961). During autumn and winter, generally males don't stay with females, while adult females share the territory among themselves with a stable hierarchy, dedicating themselves to positive social interactions such as allo-grooming. This behaviour consists in licking or gnawing a companion, where adult rabbits spend, respectively, 4% and 5% of the day in these activities (Luzi et al., 2009).

As for reproduction, as already mentioned, under Mediterranean conditions, it takes place between November and April; this is also influenced by environmental factors and social rank. In fact, testicular and ovarian development are correlated with vegetables availability; testicular function is also correlated with environmental temperature and water availability, and a positive photoperiod increase reproductive performances (Gonçalves et al., 2002; Theau-Clément et al., 2008; Mousa-Balabel and Mohamed, 2011); a high social rank guarantees a higher fecundity in females and a lower mortality in their litters (von Holst et al., 2002). Behaviours observables during mating season are: anogenital sniffing, jumping over the back, parallel hopping and circling around each other (IGN, 2011; González-Mariscal et al., 2022).

Maternal behaviours. In nature, during the last days of pregnancy, females prepare their nest, either by digging in existing tunnels or by digging suitable tunnels, and arranging leaves, dry grass, and their own fur (pulled off from their ventrum) as litter. After kindling, the mothers eat the fetal (freeing the kits) and umbilical cords and the placenta (Luzi et al., 2009). Mothers keep their kits inside the nests, whose entrances are covered to protect offspring from predators and are opened once or twice a day for three minutes by the mothers to milk the young (Maertens and Coudert, 2006; Dal Bosco et al., 2019). Although pregnancy hormones play an important role in initiating maternal behavior, they are not sufficient to consolidate and maintain it after birth. Intense contact between the mother and the offspring in the immediate postpartum period, including licking them and ingesting amniotic fluid and placenta, is essential to consolidate her maternal responsiveness. Removing the offspring immediately after birth prevents most rabbits from exhibiting maternal behaviors. Stimulation during suckling and contact with the litter are essential to maintain maternal responsiveness (González-Mariscal et al., 2022). In the second post-weaning week, the females leave to kits their caecotrophs as feed (Maertens and Coudert, 2006). As for nesting time (time spent inside the nest), females spend 12% of their day-time, that can reach a peak of 80% during the pre-kindling period (Luzi et al., 2009).

Kits, staying huddle in the littler, can maintain a good body temperature and begin to develop first social interactions (Maertens and Coudert, 2006; González-Mariscal et al., 2016). In the first weeks of life, especially in the first one, kits compete with each other for the ingestion of milk, and this will subsequently establish a weight hierarchy, since those who access the milk more easily have a greater growth. As already mentioned, in the second week of life they begin to ingest the caecotrophs left by the mother and can distinguish them from those of the other does (Maertens and Coudert, 2006). It also emerged that the diet of the does influences the future food preference of kits, whatever the foods ingested, this is then added to the individual choice of weaned rabbits (Altbäcker et al. 1995).

1.3. Evolution and Characteristics of Conventional Rabbit Husbandry

The first rudimentary farming of rabbit dates to Roman times, in which wild rabbits alongside with other wild species like hares and deers, were kept in enclosed spaces, called "*Leporaria*", where they were hunted (Luzi et al., 2009). Then, the first semi-intensive farming began in the Middle Ages (Lebas et al., 1997), when monks kept rabbits in spaces equipped with floors, for example inside the corridors of the monasteries or paved leporarias, feeding the animals with vegetables and green fodder, providing straw bedding, rearing them in groups and keeping natural matings (Lebas et al., 1997; Luzi et al., 2009). This type of farming allowed the rabbits to get used to the presence of men, starting the domestication process.

Until 20th century rabbit farming was rural and the meat obtained was destined for self-consumption; the management was similar to what developed by the monks, with the addition, from the 18th century, of the use of cages as housing system (Zoccarato, 2008). After the economic boom of 50s-60s, as for the other livestock, the rabbit farming began to turn into industrial production with the introduction of: individual housing; artificial insemination based on the new knowledge about the physiology of reproduction in rabbits; controlled photoperiod; feeding with complete pelleted diets suitable for the needs of the various physiological phases (Lebas et al., 1997; Mirabito et al., 2005b; Zoccarato, 2008).

Then, from 70s, genetic selection started also in rabbits, producing so-called commercial hybrids (Finzi and Gualterio, 2008), where genetic centers exploit heterosis by making a pushed selection within breeds (Gamberini, 2009). In fact, the pure breeds were medium in size for the female lines, i.e., New Zealand White and Californian, and heavy in size for the male lines, usually Flemish Giant

(EFSA, 2020). Regarding to male lines, the selection aims at improving average daily weight gain, feed conversion ratio, weight at slaughtering and slaughter yield, organoleptic quality of meat and resistance to diseases. As for the female lines, the focus is on fertility rate, interpartum, prolificacy, number of weaned kits, litter size and weight, less aggressiveness towards other rabbits, maternal care, milk production for kits (quantity and quality, and a sufficient number of nipples to feed the whole litter), less pre-weaning mortality of kits and resistance to disease. Selection can also focus on certain types of traits can lead to some negative effects, such as greater susceptibility to certain diseases, and less adaptability to different environmental/housing conditions (Sánchez et al., 2012; Rosell and de la Fuente, 2018).

1.4. Domestic Rabbit Welfare and Alternative Husbandry in Farms

1.4.1. Animal Welfare, definitions and on farm measurements

There are different definitions of animal welfare (Appleby and Hughes, 1997; Dawkins, 2003; Fraser, 2008; Hemsworth et al., 2015; Mellor and Beausoleil, 2015), but all of them could be summarized in the “five freedoms” proposed in the Brambell Report (1965) and later taken up by the Farm Animal Welfare Council (1979), who consider welfare in terms of freedoms as stated hereby:

1. Freedom from hunger and thirst; it's necessary a correct administration of feed and water, to prevent prolonged hunger and thirst;
2. Freedom from discomfort; comfort around resting, thermal comfort, ease of movement;
3. Freedom from injury, disease and pain; induced by management procedures;
4. Freedom to express normal behaviour; in the case of rabbits, the exploratory, locomotory, self-care and social behaviours;
5. Freedom from fear and distress.

These five freedoms have been revived, updated, and kept up to date by the World Organisation for Animal Health (WOAH) with the latest version in 2023 in their manual: Terrestrial and Acquatic Animal Health Codes.

Then, when assessing risks for animal welfare (EFSA, 2012), “A welfare consequence is the change in welfare that results from the effect of a hazard or factors influencing welfare”. As for welfare consequences in rabbit farms (EFSA, 2020), a total of 20 welfare consequences have been identified (Table 1).

Table 1. *List of 20 welfare consequences grouped in behaviour- and health-related welfare consequences.*

Behaviour-related welfare consequences	**Health-related welfare consequences**
○ Restriction of movement	○ Prolonged hunger
○ Resting problem	○ Prolonged thirst
○ Inability to express maternal behaviour	○ Pododermatitis
○ Inability to express positive social behaviour	○ Locomotory disorders
○ Inability to express gnawing behaviour	○ Skin lesions
○ Occurrence of abnormal behaviour	○ Respiratory disorders
○ Fear	○ Gastrointestinal disorders
	○ Skin disorders
	○ Reproductive disorders
	○ Mastitis
	○ Neonatal disorders
	○ Heat stress
	○ Cold stress

According to EFSA (2020), currently, the following risk factors have been identified with respect to the most relevant welfare consequences as for behavior in rabbit farms:

- Housing system: cages with insufficient available area cause a restriction of movement, i.e., the inability to do three consecutive hops, and resting problems, because rabbits are unable to lie fully stretched;
- Individual / Group housing: individual housing, without even visual and olfactory contacts, causes the inability to express positive social interactions (sniffing and grooming). On the other hand, there are also welfare consequences in the group housing system, especially for rabbit does, due to aggressiveness, indeed skin lesions and wounds, but also fear and resting problems can be detected;
- Absence/Shortage of structural and environmental enrichments: rabbits show abnormal behaviours, i.e., stereotypes, such as repetitive chewing and licking at the cage bars, whereas the lack of objects such as roughage or gnawing sticks implies the inability to express gnawing behaviours.

In fact, in rabbit farms, injuries and pain are induced by aggression by conspecifics, especially in the case of does, neglected pathologies (EFSA, 2020). The most possible causes of fear and distress for rabbits are fear-distress caused by narrow spaces, inability to express species-specific behaviours and possible aggressions by conspecifics in the case of group farms.

To evaluate the degree of animal welfare in a farm, several animal welfare evaluation protocols have been developed in several livestock species, whereas less data are available for rabbits. At the European level, firstly a COST (European Cooperation in Science and Technology) Action titled "Multi-facetted research in rabbits: a model to develop a healthy and safe production in respect with animal welfare" identified key welfare indicators in the assessment of rabbit housing (Hoy, 2009):

1. Mortality: no or low (unavoidable) mortality;
2. Morbidity: pathologies ("internal diseases", infectious factorial diseases); injuries; the morbidity should be low and unavoidable;
3. Physiology: hormone levels, heart rate variation, immune reactions; the physiological parameters should be in the species-specific standard;
4. Behaviour: ethogram, reaction to behavioural tests - species - specific behaviour;
5. Performance (production): growth, feed conversion, fertility rate; the performance should be on a high level.

Animal-based measures (ABM) are recommended to assess the welfare state of individual animals which should permit us to measure the state of the animal with respect to (de Jong et al. (2011):

- Good feeding: absence of prolongated hunger through body conditions score or percentage of emaciated rabbits at the slaughterhouse, and absence of prolongated thirst through resource-based measures (number of drinking points per rabbit, functioning of the drinkers, cleanliness of the drinkers and height of drinkers);
- Good housing: comfort around nesting (e.g., fully stretched lying), thermal comfort (respiration rate and red ears) and ease of movement (e.g., count of consecutive hops);
- Good health: absence of injuries, diseases and pain induced by management procedures;
- Appropriate behaviour: expression of social behaviour (i.e., scoring injuries and wounds), expression of other behaviours, good human-animal relationship (i.e., human approach test) and positive emotional state (e.g., fear for new objects).

Recently, EFSA reviewed protocols and indicators available for all farmed animals which outlines the scarcity of tools in rabbis (EFSA, 2023).

In matters of legislation and regulations there is Directive 98/58/EC about minimum standards for the protection of farmed animals in general, followed by specific ones for the protection of calves, pigs, laying hens and broiler chickens. Regarding rabbits, there are currently no specific directives, however some European Union countries, such as Belgium, the Netherlands, Hungary and Italy between 1998 and 2019 have drafted their own legislation or recommendations about housing of farmed rabbits, recommending the group-housing, with the aim of making rabbits' conditions similar to natural ones (Gerencsér et al., 2019; EFSA, 2020). In Italy, the Ministry of Health issued in 2014 and 2021, specific guidelines for the welfare of rabbits on farm that include the minimum dimensions for cages, enrichments and feeding (Italian Ministry of Health, 2014 and 2021).

Nevertheless, in 2017 the European Parliament approved a resolution aimed at promoting the transition from conventional cage systems to alternative housing systems that respect animal welfare (European Parliament, 2017). European citizens are increasingly demanding conditions for the welfare of livestock on farms, in the case of rabbit farming, they ask, especially for rabbit does, to apply the group housing system and more space to guarantee interactions with the like and to be able to move freely as wild rabbits do (Morgan and Tromborg, 2007; López et al., 2020). For these reasons, a group of NGOs launched a European Citizens' Initiative (ECI) named "End the Cage Age", where they ask to European Union to permanently abolish the use of cages for all farmed animals, including rabbits, in Europe (EU Commission, 2021). After a collection of over a million signatures from every Member States, in 2021 the European Commission welcomed the ECI and propose to phase out the use of cages for all the animal species and categories referred to in the initiative, including rabbits. The proposal will be part of the revision of EU animal welfare legislation expected within next 2027.

1.4.2. Housing Systems

In rabbit farms, there is a clear distinction between production categories of farmed rabbits, based on sex, age and physiological phase, for which different housing systems or management conditions can be applied. In details, the productive categories are:

- Breeding bucks: as for conventional commercial systems, they are usually farmed in specialized centres, and they are purebred in the case of three-way crossings or commercial hybrids in the case of four-way crossings; industrial farms apply exclusively the artificial insemination, so the males are used for the production of fresh semen;
- Reproducing does: in conventional farms females are bred individually in cages. Rabbit does can be artificially inseminated after synchronizing their oestrus. Pregnancy lasts 30-31 days; their litter usually comprises 8-10 kits, for about 30 days, and as in nature, even in breeding most does nurse their kits only once a day during 3-4 min (Hoy et al., 2000); after 16-18 days, kits begin gradually to eat solid feed and start their weaning;

- Growing rabbits: after weaning, 28-35 days of age depending on farm management, young rabbits (both sexes) can be moved to new enclosures where to stay in couples (bicellular cages) or they can remain in the cage where they were born while their mother is transferred into another pen (dual-purpose cages). They remain in these cages until slaughtering age (70-90 days depending on genetics and systems).

In conventional housing systems, cages with different sizes are used for the various production categories of rabbits. Normally, pregnant and lactating rabbit does are housed individually in wire mesh cages with variable length between 87 and 102 cm, width between 38 and 46 cm and height between 32 and 35 cm, with a total available surface between 3300 and 4700 cm^2 (Szendrő et al., 2019). Cages are equipped with nests for kits, internal or external to the pen itself, which allow free access inside or only at certain times of the day (programmed lactation system). The kits remain inside the nest for the first two weeks of age, and then go out and stay with the mother.

The young rabbits for replacement are housed in special individual cages, smaller than those used for does, with length of 38 cm, width between 45 and 50 cm and height of 35 cm with a walkable surface between 1700 and 1900 cm^2. These cages are also used for inseminated but not pregnant does.

Growing rabbits are housed in pairs in bicellular cages in wire mesh cages with a length between 40 and 45 cm, width from 24 to 28 cm and height between 28 and 30 cm with a total available surface for two rabbits between 960 and 1260 cm^2, the individual cages are grouped in batteries even on several floors (EFSA, 2005; Gamberini, 2009; Szendrő et al., 2019). In alternative to bicellular cages there are group housing pens, with the same dimensions as cages for young or non-pregnant females (38 x 43,5-66 x 28-41 cm, respectively width, length and height, and an available surface of 1650-2510 cm^2), and the so-called dual-purpose cages, which in practice are the conventional cages of does, with the difference that kits, once weaned, remain in their "origin pen", while the does are transferred to disinfected cages to begin a new cycle (EFSA, 2020).

In some countries there is a transition to alternative housing systems for does and growing rabbits. In particular, the alternatives of standard cages (also called California if they are multi-levelled) are the WRSA cages and parks (Figure 4):

- WRSA cage or welfare cages: following the WRSA (World Rabbit Science Association) guidelines, it has a greater height (up to 60 cm) than the standard cage to allow the rabbits to stand up completely with the front legs without touching the ceiling of the cage, partially plastic floor (with footrests) to preserve the health of fore legs and is equipped with an elevated platform to increase the walkable surface and allow rabbits to jump and make more movements; it is possible to find environmental enrichments such as gnawing woods. Minimum measures for each elevated platform are: 25 cm of height under the platform, 20 cm of width and 900 cm^2 of free area (Italian Ministry of Health, 2021).

- Park: recently introduced in EU and required by Belgian legislation (EFSA, 2020), characterized by the absence of the galvanized net on the ceiling, it has completely plastic mesh and larger dimensions than traditional cages for rearing animals in groups, it has the platforms and in addition also removable dividers that allow the division of the park into modules. A park needs of an elevated platform, gnawing objects, shelters (pipes or tunnels long at least 40 cm, also nests are counted as shelters) and at least 4 feeding points and 2 drinking points (Italian Ministry of Health, 2021). Minimum measures for an elevated platform are 25 cm of distance between floor and platform surface and 27 cm of width, while the surface should be between 25 and 40% of the whole available surface. As reported also by farmers, parks are the best environment to express natural behaviours: hopping, stand up and gnawing behaviours (EU Commission, 2017).

Figure 4. *Standard Cage or California (left), WRSA cage (middle) and Park (right).*

Nevertheless, as for production costs, Mondin et al. (2021) compared bicellular, dual-purpose and WRSA cages, using the price per kg of meat produced as an index; they collected data from six farms, and they found out that the slaughter weight / m^2 is not significantly affected across the different systems, while drugs use (so the costs) was lower in WRSA cages than bicellular cages, as energy and feed costs, on the other hand, reproduction costs (due the farm effect) and cage costs (due to high purchasing price) were higher than the other systems.

1.4.3 Welfare of rabbits in the different housing systems

Different considerations about the welfare of the different categories of rabbits can be done with respect to the different housing systems and the structural characteristics of the housing systems as discussed hereby.

Indeed, EFSA (2020) assessed the welfare level of rabbits and the severity of different welfare consequences in them in different housing systems using the "Knowledge Elicitation Process" (EKE), i.e., consulting experts to gather information about various welfare consequences obtained from their experience as they are not available in the scientific literature. After that, a score was assigned for each welfare consequence, which was then added up to obtain an overall welfare impact score, that have a range of 0-9. Six housing systems were evaluated: conventional cages (with plastic footrests; standard cages for reproducing does, dual-purpose cages and bicellular wire cages are included), structural enriched cages (that are equipped with elevated platforms and plastic footrests; WRSA and enriched dual-purpose cages are included), elevated pens or parks, floor pens, outdoor and partially outdoor systems, and organic systems. The production categories considered were rabbit does, kits and growing rabbits. EFSA (2020) concluded that it is 66-90% probable that the welfare of reproducing does is lower in conventional cages than the other systems, which do not have significant differences between them, and the restriction of movement is the welfare consequence with highest impact score (0.87), followed by the lack of gnawing materials and the hunger. EFSA (2020) concluded that it is 66-99% probable that growing rabbits' welfare is lower in conventional cages than the other systems, while parks allow a higher welfare, and the restriction of movement is the welfare consequence with highest impact score (1.29), and with the lack of gnawing materials and the resting problems contributes more to the overall welfare impact score.

EFSA's conclusions (2020) about the six housing systems are that for rabbit does and growing rabbits the welfare consequences related to behavioral restrictions are more evident in conventional cages, enriched cages, and parks, on the other hand, these systems have less health problems than the other three (floor pen, outdoor system and organic). Indeed, different factors are playing a role in the different housing system in affecting rabbits welfare. The major factors that have been identified in the EFSA Opinion and then addressed in the contributions of the present PhD Thesis are discussed hereby.

Firstly, conventional husbandry is associated to high hygiene for all categories (thanks to the wire-mesh cages) and the reduction of pseudo-pregnancies for reproducing does (due to individual housing and artificial insemination), attacks against kits by other does and the overloading of the nests. On the other hand, typical behaviors of the species and social behaviors are reduced or eliminated (Trocino and Xiccato, 2006), while abnormal behaviours can appear, such as bar-biting and hair chewing (Gunn and Morton, 1995; Morgan and Tromborg, 2007) and locomotor behaviors can be reduced (Chu et al., 2004).

As for the floor, several studies show that the use of footrests (Figure 5) reduces pododermatitis in reproducing does (de Jong et al., 2008; Rosell and de la Fuente, 2009), however there is the problem of accumulation of feces in the cage. A full plastic floor (Figure 6 and 7) is preferred by rabbits (Princz et al., 2008b), but can be associated to great hygiene and health problems for growing rabbits (Trocino and Xiccato, 2024). Alfonso et al. (2011) showed how long reproducing does stay on footrests compared to wire-meshes (in the study: 81.7% of the time during lactation and 52.6% of the time during gestation).

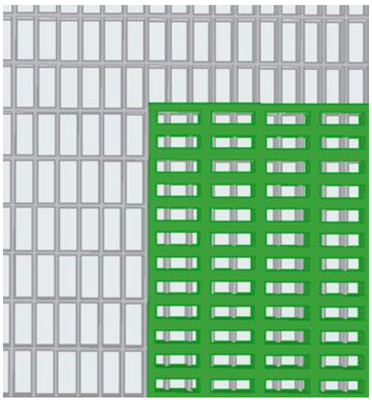

Figure 5. *Footrest on the wire-mesh floor.*

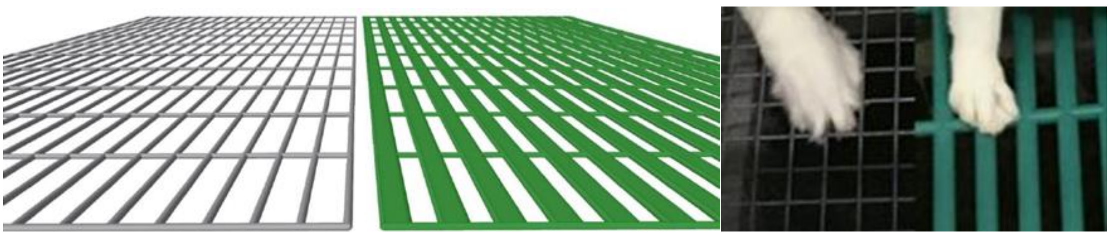

Figure 6 and 7. *The plastic mesh (green) allows a greater support surface than the wire-mesh floor (grey).*

As for other types of cage flooring, in a preference test between solid and straw-bedded floors and plastic floors, the does preferred the second one (Ruis, 2006; Szendrő et al., 2013). Morisse et al. (1999) also observed that growing rabbits preferred wire-mesh floor to straw litter for most of the day, in particular for resting, whereas budget time was not affected by the floor type. On the other hand, production performances were negatively affected by straw litter. Between wooden slats and plastic floor, growing rabbits housed on the former type rested more in the crouched position and less in stretched position, and performed less allo-grooming, bar-biting, and running than the latter group, besides showing higher hair corticosterone levels compared to the latter animals (Trocino et al., 2018).

Dal Bosco et al. (2002) compared housing in conventional bicellular cages, straw-bedded pen and the wire-netted pen and found that in the cages growing rabbits had more feed intake and less locomotor behaviours than in the pens; on the other hand, in the pens there were more social and locomotor behaviours than in the cages, but the growth rate, the feed conversion ratio and mortality were higher. Then, the straw-bedded pens gave the poorest results for performance due to the ingestion of straw and the direct contact with excreta; on the other hand, there were more locomotor and social behaviors than the wire-netted pens.

As for alternative systems, Mattioli et al. (2016) compared mobile arks with conventional bicellular cages, finding out that rabbits in mobile arks (placed outdoor on alfalfa pasture) showed a higher locomotion and, at the meat level, a higher content of antioxidant molecules (vitamins, polyphenols, PUFA) and omega 3 than in the cage, while reducing fat content in Longissimus lumborum muscle.

With regards to abnormal behaviors and the effect of enrichments, one of the key points for improving rabbit welfare is the possibility of allowing them to express the typical behaviors of the species, to reduce stereotypies and self-destruction behaviors (e.g., bar-biting and hair chewing). From this point of view, both structural and environmental enrichments have been devised. The structural enrichments can be considered as elevated platforms, tunnels, and PVC pipes. As for platforms, they are usually 30-40 cm above the floor; they can be made of metal or plastic, where the Spanish legislation for the protection of rabbits used in experimentation and other scientific purposes indicates that it must not cover more than 40% of the floor, and that it must have a width of 55-60 cm and a depth of 25-35 cm.

Elevated platforms allow to increase the behavioral patterns of the does (e.g., jumping or standing on the hind legs) (Alfonso-Carrillo et al., 2014); it also increases the available surface, and seems to reduce aggression during groupings in the semi-group system (Rommers et al., 2014a). As indicated by Mikó et al. (2012), does prefer plastic platforms over wire ones. According to Alfonso-Carrillo et al. (2011), in pens equipped with platform and footrests the does spend more time on these two than the wire-mesh (respectively 25.1% and 51.8% during lactation and 20.8% and 44.6%

during gestation). Moreover, platforms can allow females to separate from kits when they annoy her (Mirabito, 2003; Alfonso-Carrillo et al., 2014).

On the other side, as for platform, a critical point of the platform can be the lower hygiene, as feces and urine fall on the animals below, and feces can accumulate, which can increase the occurrence of pododermatitis and bone fractures (Olivas et al., 2010; Alfonso-Carrillo et al., 2014; López et al., 2019).

Confronting pens without any type of platforms, pens with wire-mesh platforms or plastic-mesh platforms, in the latter cases there were two levels of platforms (25 and 50 cm from floor) (Martino et al., 2016; Gerencsér et al., 2016; Farkas et al., 2016; Matics et al., 2018), it was observed that platforms ensured higher locomotion than in the pens without them, but in the meat there was a lower content of antioxidants; however, overall, the presence of growing rabbits was concentrated on the floor, especially in front of platforms instead under them; taking into account only the platforms, rabbits preferred the plastic-wire type, especially the ones of second level; about productive performances, there weren't significative differences between pens (also Lopez et al. 2020 stated that platforms don't influence the productive performances), while as regards the meat (especially the longissimus lumborum muscle), it was observed higher content of retinol, γ-tocotrienol, linoleic and linolenic acids in "plastic-mesh platform" group, while in "no platforms" group there was higher content of α-tocotrienol, α-tocopherol and n-3 PUFA.

PVC pipes, on the other hand, allow the does to hide during attacks in group housing systems, reducing serious injuries (Rommers et al., 2014a). Regarding the growing rabbits, the tubes, from a paper by Trocino et al. (2019a), didn't change the behavior of animals, moreover, the average daily gain was lower for those who benefited from this enrichment.

Environmental enrichments can be represented by gnawing blocks of various materials, straw, gnawing sticks on the floor (not recommended due to poor hygiene, as indicated by López et al. 2020) or hanging, hanging ropes, etc. Interaction with an object is an important stimulus in rabbit exploratory behavior expression, particularly in small-size groups (Zucca et al., 2012). Gnawing materials are the most used as environmental enrichment (Trocino et al., 2019a), and their presence can reduce the stress of rabbits by reducing stereotypies and bar-biting (Verga et al., 2004; Princz et al., 2007), as well as aggressions (Princz 2008a). However, Bozicovich et al. (2016) concluded that the availability of gnawing blocks increases aggression, which can depend on the specific rearing conditions which can increase competition for resources among animals and thus aggression.

Roomers et al. (2014b), testing straw, compressed wooden block, pinewood stick and the combination straw + pinewood stick as gnawing materials, observed that most of the does consumed straw and wooden block for longer, while pinewood sticks were minimally consumed (respectively, these gnawing materials were preferred by 24, 11 and 4% of does, and consumed during 4, 2 and 0.1% of observed time). However, straw does not reduce aggression injuries in rabbit does (Roomers et al., 2014a).

Maertens et al. (2013) experimented three types of gnawing blocks, with both does and growing rabbits: all 3 types of gnawing blocks were composed by wheat, molasses, and trace elements, then the first type had the wood mash added, the second a mix of wood mash and chicory pulp, and the third a mix of wood mash and inulin syrup. With both does and fattening rabbits, gnawing blocks with only wood mash were preferred, followed by wood mash + chicory pulp (11 vs 6.8 g / d per cage in the case of does, and 7 vs 3.9 g / d per rabbit in the case of fatteners). However, the first type of block decreased production performance, so the second type would be preferred. The research revealed an increase in locomotor behaviours and in the feed and water intake in the does that have gnawing blocks.

1.4.4 Group Housing

It was observed that wild rabbits spend 54.9% of their time, while domestics spend 30.6%, in groups of 2 or 3, and most of this time (80.4% and 65.3%, respectively wild and domestic rabbits) they spend in voluntary body contact (Maertens and Coudert, 2006). On farm, while on the one hand individual breeding involves the absence of positive interactions with conspecifics, such as sniffing and allo-grooming, on the other hand breeding in groups can lead to problems of aggression especially in adult rabbits (or those who have exceeding 60 days of age) and consequent skin injuries, and a too high stocking density also leads to resting problems due to the impossibility of the single rabbit to lie down completely. In bicellular cages compared to individual housing, the bar-biting phenomenon is reduced or disappears, furthermore the animals help each other to warm up in winter (and the higher body temperature observed compared to that of the individually bred does confirm this) and manifest social behaviors, such as huddling together, grooming, and nuzzling each other (Burn and Shields, 2020).

As already mentioned, one of the major requests of society is to be able to raise rabbits in groups as it occurs in nature. However, under farm conditions, the weak point of the group system consists in aggressive interactions, also called agonistic behaviors.

As far as growing rabbits are concerned, group housing implies a need for larger spaces in alternative systems compared to conventional cages, which can mitigate movement restrictions. As previously stated, WRSA cages can house up to 5-6 growing rabbits, while parks can host up to 32-36 individuals. Currently, the maximum stocking density used between Member States in EU for growing rabbits is 45-50 kg/m^2 in conventional cages, 40 kg/m^2 in enriched cages (i.e., WRSA and enriched dual-purpose cages) and 30 kg/m^2 in park pens (EU Commission, 2017). Agonistic behaviours in growing rabbits increase jointly the age reaching the peak at 80 days of age, and these could be accelerated due to accelerated sexual behaviours (for example in case of presence of adult females) and/or withing larger groups (Maertens and Coudert, 2006). EFSA (2005) recommended to keep groups of 7-9 rabbits, possibly from the same litter, with 16 rabbits / m^2 (40 kg slaughter weight / m^2) as an optimal density stock to reduce aggression and distress, and keep a good growth rate (Luzi et al., 2009; EFSA, 2020). Trocino et al. (2015) found out a high rate of injuries from aggressions in pens with 16 animals m^{-2} than in pens with a density of 12 rabbits m^{-2} (26.2% vs. 8.2%, $p \leq 0.001$), moreover the aggressiveness was recorded more among males than females (25.8% vs 11.3%, $p \leq 0.001$). With the increase of stocking density (from 12 to 16 rabbit/m^2), the resting in stretched position and the self-grooming time decrease, as the percentage of rabbits that spontaneously entered the arena during the open-field test decreased, while it was observed an increase in the percentage in resting during the day, as the resting in crouched position (Trocino et al., 2018). Dual-purpose cage system prevents the mixing of growing rabbits' groups, allowing to reduce fights at the beginning of sexual maturity (EU Commission, 2017).

As for the reproducing does, the problem of aggression is the biggest obstacle that does not allow females to be reared in a group in a confined space without having side effects such as weight loss, injuries, termination of pregnancy and even death. The agonistic behaviours are towards the mates and the kits, when present, with consequent high levels of stress (Szendrő et al. 2013) and high levels of mortality of kits, due to attacks by other does, since they have access to other nests (Ruis and Coenen, 2004), the kindling in the same nest of two-three litters, and, then, for kits competition for maternal milk (Mirabito et al., 2005b; Cervera et al., 2016). The negative interactions among does can be divided into different behaviors, as classified by Graf in 2010 (Table 2).

Table 2. *Description of agonistic behaviors as indicated by the ethogram proposed by Graf (2010).*

Agonistic Behaviours	**Description**
One-Sided Aggressive:	
• Boxing	The rabbit pushes a conspecific with its muzzle or forelegs
• Biting	One rabbit grab another with its teeth and pulls its head back; occasionally, biting can only be inferred because that biting animal retains a tuft of hair in its snout
• Threatening	Intense movement of the head in the direction of one conspecific; the rabbit possibly opens its mouth, representing a threaten to bite
• Attack	Abrupt race towards a conspecific
• Chasing	Two animals move very quickly, less than two meters one after the other over a distance of at least 4 quick hopping jumps
• Escape	Jumping or running being chased by another rabbit or in reaction to approaching another rabbit
• Mounting	Attempts to mount
Two-Sided Aggressive:	
• Ripping	An animal bites into the fur of another and throws itself to one side and kicks the other with his hind paws; sometimes both of these are done by both animals at the same time
• Jump up	Two animals jump in the direction practically at the same time up towards each other and kick in the air with their hind paws against each other; this behavior represents an intense argument is repeated several times or goes into ripping
• Carousel	Two animals stand antiparallel, each with its head biting the back of the other, forming a circle

Remembering that in nature, females live in small groups and generally give birth individually in burrows or at most divide the nest into small groups (2-3), there is not much promiscuity in the days before or after the birth. Similarly, several studies have tried to understand if there are differences in terms of aggression with respect to the size of the group. Martínez-Paredes et al. (2019) concluded that in very large groups (16-24 does) injuries are more frequent than in small groups (2-4 does). Matics et al. (2017) performed a preference test with groups of 4 nulliparous rabbits, observing that they prefer to be alone or in the company of one rabbit. Also, Dal Bosco et al. (2020) concluded that does spend more time alone with their litters than in the company of other does (50.39% vs 49.61% in the first experiment and 71.90% vs 28.10% in the second experiment). Does in groups of 8 rabbits showed a higher agonistic behaviour than in groups of 4 rabbits, and groups of 4 rabbits showed a higher frequency of boxing and chasing than in groups of 2 rabbits (Buijs et al., 2016; Zomeño et al., 2017). In semi-natural conditions, Rödel (2022) reports that agonistic behaviors between rabbit does increase as group size increases, with a more homogeneous age (social-rank tends to rise with age, therefore in a group with heterogeneous age the social-rank between the does is more stable and better structured) and if the births between the various companions are concomitant (also because a does gets too close to a litter of others, and then she will be attacked by the mother). Furthermore, the number of kits born for does decreases as the stocking density increases.

1.4.4.1 Grouping Times

Several studies evaluated a continuous group housing, i.e. maintenance of the does together for the entire production cycle, and found the strong problem of aggression. In continuous group housing, agonistic interactions and the incidence of injuries are high (Andrist et al., 2013; Pérez-Fuentes et al., 2020), as well as the pseudopregnancies due to mounts among does (Rommers et al., 2006; Mugnai et al., 2009; Andrist et al., 2013). On the other hand, productivity is lower than in the conventional individual housing system (Mugnai et al., 2009). Furthermore, group management increases aggression, especially towards low-ranking rabbits (von Holst et al., 2002), and therefore chronic stress: Szendrő et al. (2013) measured the amount of corticosterone in the feces, detecting a higher amount in the group housing than in those kept the individual cages (175 nmol / g vs 54-61 nmol/g). The mortality of kits was higher due to aggression by other does (Szendrő et al., 2016), especially by high-ranking does against the litters of lower ranking does (Rödel et al., 2009). The cases of attacks on does and kits are greater in younger rabbits (Ródenas et al., 2019) and in cases where a substitute does is included in the group (Andrist et al., 2012). Pseudopregnancies are probably one of the causes of the reduction in the fertility of does (Mirabito et al., 2005a; Rommers et al., 2006), as well as the reduced number of kits born.

With the results obtained in the various studies on continuous group farming, researchers concluded that currently this system is not recommended and should be avoided (Szendrő et al., 2019; Dal Bosco et al., 2020).

A possible alternative to the continuous housing group system is the "part-time" system, in which does are grouped only at certain times during the production cycle. This part time systems can be managed using pens equipped with movable walls that can be removed during grouping and put back during separation. The does are usually separated by 3 days pre-partum until the kits leave the nest (around 18 days post-partum) (Andrist et al., 2012; Machado et al., 2016; Cervera et al., 2017). Rödel et al. (2008) observed (in semi-natural conditions) that separating the does before kindling and up to 12 days postpartum brings benefits both in the moments of grouping and in those of separation. In fact, litters are protected from the attacks of the other does where the infanticides are usually concentrated in the first 10 postpartum days. Nevertheless, the moment in which groups are formed (e.g., after kindling or during lactation) affects the level of aggressiveness of the does (Zomeño et al., 2017, 2018).

In part-time systems, the mortality of kits is still higher than in the conventional system (Cervera et al., 2017) and the problem of aggression is still unsolved. Munari et al. (2020), comparing grouping time of 12, 18 and 22 days, observed that the number of attacks did not change, whereas aggressive behaviors among does are higher in 12-day system compared to the latter ones. Rommers and De Greef (2018) found that in a part-time system with 5 does the injury rates increase from 4 days after group formation (23 days post-partum) (34%) to litter weaning (36 days post-partum) (53%), on the other hand, Zomeño et al. (2017) observed that aggressions are greater at the first group formation (8 days pre-partum) than at regrouping (at 18 days post-partum).

Dal Bosco et al. (2019) compared the effects of individual systems (C), continuous group housing (C1) and part-time group housing (separation between four days before and one week after kindling) (C2) on welfare, reproductive performance, and global efficiency: does on C group showed the highest presence of stereotype behaviors; in C1 group there were more aggressions than in C2 group (22% vs 17%); C group does had the best productive and reproductive performances (i.e. fertility, number of kits alive on kindling and weaning), whereas C1 had the lowest performances.

Finally, part-time group housing systems have shown some potential, but their adoption under farming conditions cannot yet be recommended due to the high aggression level and, consequently, the rate of injured does measured after each regrouping. More research is necessary to limit harmful and painful behaviors among does (Szendrő et al., 2019).

1.4.4.2 Group Management

A big problem for rabbit does kept in group is the high rate of infanticides by the does against kits not theirs. Ruis (2006) proposed an electronic recognition system for opening nest boxes, in order to allow only to the mother of the kits, and in the experiment the survival rate of the young was improved, however the pseudopregnancies and the fights between does were not resolved, and the kindling rate and weaning weight remained low. Mirabito (2003) and Alfonso-Carillo et al. (2014) concluded that the platform allows doe to "escape" from their kits when, once out of the nest box, they begin to annoy the mother for feeding, however in some experiments, such as that of Mikó et al. (2012), even the kits went up on the platform (from the 17th day of age, the moment of leaving the nest, until weaning). Thus, Mirabito et al. (2005b) had proposed to place the feeders for the kits on the ground, and exclusive feeders for the does 32 cm from the mesh, showing how this has positive repercussions on the growth and welfare of the kits.

In commercial farms losses of reproducing does are frequent (e.g., by death, illness or severe injuries), which leave vacancies in the pens. This leads to the addition of new does, the so-called "intruders", in an existing group, composed of the so-called "residents", causing territorial aggressions, resource competitions and the re-establishment of a hierarchy (Graf et al., 2011). Morton et al. (1993) and Love (1994) recommended regrouping unfamiliar rabbits in a novel pen, a neutral space without does' odor markings, used for example to communicate information like territory ownership or dominance status (Bell, 1981; Marai and Rashwan, 2003). In this regard, Graf et al. (2011) compared the insertion of new does in pens with already formed groups ("home" group) and in clean and disinfected pens together with the does of other groups ("novel" group). General activities (e.g., grooming, resting, and feeding behaviors) did not differ between two treatments, as well as on the number and duration of agonistic behaviors, however, the number of injuries and the body temperature (used to monitor the stress of the does) on the first day after regrouping were greater in the “novel” group compared to the “home” group. Considering these results, it’s preferable to insert the unfamiliar does in pens with already established groups.

1.5. References

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2. AIMS

This thesis is placed in a context in which society is paying more and more attention to the welfare of farmed animals. This increase in awareness is driving a shift towards more animal-friendly farming systems. There is growing pressure from consumers and animal rights activists for more attention to be paid to the welfare of farmed rabbits. Although several studies have been carried out to improve their welfare and health, there are still many unanswered questions, particularly in relation to the types of housing that can contribute to good health and appropriate behaviour. Precisely the type of accommodation is a much-debated topic. Although it is true that the most advanced cage-free housing systems (the parks) have been an important step for the increase in welfare as they have allowed greater motor activity, the interaction with a wider range of environmental enrichments and breeding in groups, it also brings with it some critical issues as regards the rabbit does which present a latent aggression between individuals. Additionally, also in growing rabbits, groups housing can lead to health problems caused by non-optimal hygiene especially if associated with enrichments such as not suitable platform.

In this framework, this Ph.D. thesis specifically evaluated:

- Use of gnawing hay blocks: effects on productive performance, behavior and reactivity of growing rabbits kept in parks with different sex-group compositions (first contribution);

- A pilot study about on-farm assessment of health and welfare in rabbits kept in different housing system (second contribution)

- Movement Restriction in Reproducing Does in a Part-Time Housing System: Effect of Group Size and Grouping Time (third contribution).

3. Contribution 1: Use of gnawing hay blocks: effects on productive performance, behavior and reactivity of growing rabbits kept in parks with different sex-group compositions.

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INTRODUCTION

In the European Union (EU), meat rabbit farming is concentrated in three member states, Spain, France, and Italy (about 85% of the EU production) (Trocino et al., 2019b; EFSA, 2020), which still rear growing rabbits in small conventional barren bicellular or dual-purpose cages (with 2 or 4–6 rabbits, respectively), and more recently, enriched cages, that is, larger and higher cages equipped with platforms (Trocino et al., 2019b; EFSA, 2020). Nevertheless, the European citizens' initiative "End the Cage Age" asks for banning any cage system for farmed animals in the EU, including rabbits, for which the European Parliament is calling for phasing out of cages by 2027 (EFSA, 2020).

Initially used in Belgium and the Netherlands, alternative housing systems such as parks (also called elevated pens) are at a developmental stage in countries that are the main producers of meat rabbits and require improvements and standardization in terms of management and equipment (EFSA, 2020). They usually consist of open-top parks, where growing rabbits can be reared in groups of different sizes (more than 8, litter size; often 30-32). Generally equipped with plastic flooring or wire net floors covered by plastic footrest pads, parks often include elevated platforms (EFSA, 2020).

In these systems (elevated pens/parks), according to EFSA (2020), the welfare of growing rabbits is "likely/highly likely to be higher", whereas it is lower in conventional bicellular and dual-purpose cages. In parks, among the five top main welfare consequences (i.e., problems) (WC) (EFSA, 2020), besides two health-related WC (i.e., skin and gastrointestinal disorders), three behavior-related ones (i.e., resting problems, inability to express gnawing behavior, and fear) need to be addressed for the successful implementation of park systems and a further improvement of animal welfare in these systems.

Resting problems can be related to stocking density, use of appropriate flooring materials, and good hygiene. Gnawing inability is known to promote abnormal stereotypic and aggressive behaviors based on studies performed under different housing conditions (Verga et al., 2004; Princz et al., 2007, 2009; Buijs et al., 2011; Bozicovich et al., 2016). However, there is a lack of consistency regarding the effects of the presence of gnawing materials on other rabbits' behaviors, as well as a lack of evidence regarding the material that can best satisfy the rabbit's gnawing motivation (EFSA, 2020). Finally, fear can be reduced by avoiding rough handling and situations that contribute to aggression (EFSA, 2020). These latter situations are more likely to occur under the conditions of collective housing in a park system, whereas the occurrence of aggressive behaviors and skin wounds has been correlated with an increase in group size (Bigler and Oester, 1996; Szendrő et al., 2009; EFSA, 2020). In this regard, whether group composition in terms of sex can affect aggression among animals is not clearly stated, since few contradictory results are available (Di Meo et al., 2003; Trocino et al., 2015; Bozicovich et al., 2016; Birolo et al., 2020), whereas rabbits are usually kept in mixed-sex groups rather than in single-sex groups.

Thus, the present study aimed to evaluate the effect of the presence of gnawing materials (as compressed hay blocks) and sex group composition (only females, only males, females and males) on the productive results, behavior, and reactivity of growing rabbits kept in a park system.

MATERIALS AND METHODS

Facilities, animals, and experimental conditions

The trial ran on the rabbit farm of the University of Padova in a closed building from October to November under a natural photoperiod (approximately 11-12 hours of lightness). Extraction fans and an automatic heating system guaranteed air quality and maintained the temperature between 20°C and 24°C.

At 31 days of age, 288 crossbred rabbits (144 females and 144 males; Hypharm, Groupe Grimaud, Roussay, Sevremoine, France) were selected from healthy litters of multiparous does (≥3 kindling) in a commercial farm, transported to the experimental farm, individually identified using ear marks and housed in 18 parks (1.28 m front/back walls × 0.78 m side walls; area: 1 m^2) with 16 animals per pen (16 animals/m^2), i.e. the typical stocking density used in commercial farms (Trocino et al., 2013). The back/front walls and the side walls of the pens (1.10 m-height) were made of galvanized wire nets (grids: 20 mm × 20 mm; diameter: 2 mm). The parks were composed by two modules of the same dimensions (0.64 m × 0.78 m) partially separated by a wire net wall (0.35 m × 1.10 m-height) between them, leaving a 0.43 m passage between the two modules. The first module represented the feeding area (0.5 m^2; left side of the pen), and the second module was the resting area (0.5 m^2, right side of the pen). The feeding area had a wire net floor (grids: 75 mm × 15 mm, diameter: 2.5 mm), four automatic nipple drinkers, and a feeder (0.60 m-wide) fixed at the front wall. The resting area had a plastic slatted floor with rectangular holes (70 mm length × 10 mm width; distance between the holes: 7 mm) and was equipped with two additional nipple drinkers.

The study was designed as a bi-factorial arrangement with three sex-group compositions per park (F: 16 females/park; M: 16 males/park; FM: 8 females and 8 males/park), combined with the absence or presence of gnawing hay blocks (replicates: 3 parks per experimental group). Hay blocks (cylinder form; length: 400 mm; diameter: 80 mm; dry matter: 89.0%; crude protein: 11.2%; ether extract: 1.4%; neutral detergent fiber: 53.1%; acid detergent fiber: 34.7%; acid detergent lignin: 9.1%) were provided *ad libitum* during the whole trial in a metal tube (400 mm length, diameter: 100 mm) fixed at the back wall of the park feeding area.

All rabbits were fed *ad libitum*, had free access to fresh water, and received a post-weaning diet (dry matter: 88.8%, crude protein: 15.4%; ether extract: 4.1%; neutral detergent fiber: 37.1%; acid detergent fiber: 19.6%; acid detergent lignin: 5.1%) from 31 to 55 days of age, followed by a fattening diet (dry matter: 88.5%, crude protein: 15.5%; ether extract: 4.4%; neutral detergent fiber: 35.0%; acid detergent fiber: 18.0%; acid detergent lignin: 4.1%) from 56 days of age until slaughter (73 days). The diets were formulated to meet the nutritional requirements of growing rabbits according to de Blas and Mateos (2010) and did not contain any antibiotics or coccidiostat.

Growth performance and health

During the trial, the individual live weight of the animals and hay block consumption (where present) of the pen were recorded once a week, and pen feed intake consumption was recorded daily. Hay block consumption was measured as the difference between initials and final weights of blocks. The morbidity and mortality rates were monitored daily. Rabbits were considered ill when they showed diarrhea and/or mucus in the feces or live weight loss during a week. The day before slaughter, the presence and severity of body injuries caused by aggression were assessed.

Behavioral recordings and observations

At 42 and 70 days of age (i.e., around half of the trial and towards the end of the trial), the behavior of the rabbits was video-recorded using infrared cameras for 24 h. Then, over two minutes per hour in each pen for 24 h at the two ages, the following behaviors were recorded and expressed as a percentage of the total observation time: time spent feeding, drinking, cecotrophy, resting (crouched body, with the abdomen in contact with the floor; stretched body, with both fore and hind legs stretched beside the abdomen in contact with the floor), self-grooming, allo-grooming, moving, running, sitting, biting/licking, and sniffing (Trocino et al., 2019). Time spent in the feeding and resting areas of the pens was also measured. Finally, the occurrence of rearing, hops, aggressive interactions, and stereotypic behaviors was recorded and expressed as the number of events per pen per observation interval (Trocino et al., 2019).

Open-field test

At 64 days of age, 108 rabbits (6 animals per park; 3 females and 3 males in the case of FM parks) were subjected to an open-field test to evaluate their reactivity towards a new environment (Meijesser et al., 1989; Trocino et al., 2018). The rabbits to be tested were randomly taken out of cages. In the case of FM parks, the sex of the rabbits was immediately checked based on the individual animal identification. The test was conducted in an arena (2 m × 2 m) with 0.80-m-high wooden walls and a plastic floor divided into nine numbered squares, placed in a contiguous room in the same stable where rabbits were kept. The total duration of each test was 10 min per animal. Each rabbit was placed in a closed wooden box (0.22 m length × 0.30 m width × 0.30 m high) connected to the arena by a sliding door. After one minute, the sliding door was opened, the number (n) of attempts made by the rabbit, and the time (latency, s) spent to enter the arena was recorded for another minute. If after this minute the rabbit was still in the box, it was gently pushed into the arena, the sliding door was closed, and the behavior of the rabbit was video-recorded for 8 min. The behaviors evaluated during the open-field test were total displacement (n), central displacement (n), movement (s), running (s), exploration (s), escape attempts (n), hops (n), standing still (s), rearing (n), grooming (s), digging (s), biting (s), resting (s), defecation (n), and urination (n) (Meijesser et al., 1989; Trocino et al., 2018).

Novel object test

At 65 days of age, reactivity towards a new stimulus was evaluated using a novel object test in all parks (Verwer et al., 2009). The novel object was a 1.5-L plastic bottle, half full of water, anchored by a cap with an iron chain, and dropped from the roof in the center of each park a few centimeters above the floor. The behavior in each park was video-recorded for 5 min, and the number of animals that touched the object was measured during the first minute (1-60 s), during the following two minutes (61-180 s) and the last two minutes (181-300 s), and then expressed as a percentage of the total number of rabbits in the park.

Human approach test

At 67 days of age, the human approach test was used to measure the animal fear level towards men in all parks (Csatádi et el., 2007; Verwer et al., 2009; Trocino et al., 2018). An unfamiliar operator opened each pen and placed a hand at the center of the pen a few centimeters above the floor (at the animals' withers' height). Rabbit behavior was video-recorded for 5 min. The number of rabbits that touched or sniffed the operator was measured during the first minute (1-60 s), during the following two minutes (61-180 s) and the last two minutes (181-300 s) and expressed as a percentage of the total number of rabbits in the park.

Commercial slaughtering

At 73 days of age, after a 4-h fasting period, the rabbits were weighed at the experimental farm. The rabbits that reached the minimum live weight requested by the slaughterhouse (2.3 kg) were caged and transported to a commercial slaughterhouse by an authorized truck, which took approximately 1 h of transport. Slaughtering took place approximately 1 h after their arrival at the slaughterhouse, where the rabbits were individually weighed, stunned by electroanesthesia, and killed by jugulation. After 2.5-h chilling, commercial carcasses were weighed to calculate the individual slaughter yield (Blasco and Ouhayoun, 1996).

Statistical analysis

Individual live weight, daily weight gain, and slaughter yield data were subjected to ANOVA using the PROC MIXED of SAS (SAS 9.4 software, SAS Institute Inc., Cary, NC, USA) with gnawing blocks (absence vs. presence) and sex-group composition (F vs. M vs. FM) as main effects with interaction, and the park as a random effect. Park feed intake and feed conversion were analyzed using PROC GLM, considering the same main effects.

The time spent on the different behaviors and the rate of rabbits in the feeding or resting areas of the parks were analyzed by a generalized linear mixed model using PROC GLIMMIX with gnawing blocks, sex-group composition, and rabbit age as the main effects of interactions. The observation time was a random effect, and data from the same pen were considered as repeated measures. An underlying Poisson distribution was assumed for all the data.

Data from the reactivity tests (open-field, novel object, human approach) were analyzed using the PROC GLIMMIX with a generalized linear mixed model considering gnawing blocks and group composition as the main effects with interaction, and pen as the random effect. An underlying Poisson distribution was assumed for the data. The percentage of animals entering the arena spontaneously in the open field test was coded as a binary variable (entering=Yes or No) and evaluated by maximum likelihood analysis using the CATMOD procedure, which used gnawing blocks, group composition, animal age, and their interactions as the main factors.

The means were compared using Bonferroni's test. Differences among means with a *p*-value < 0.05 were accepted as representing statistically significant differences.

RESULTS

Rabbit health and production results

During the trial, five rabbits died because of enteric disorders, and the other seven were discarded at the end of the trial because of their low live weight (< 2.3 kg). In details, in the parks without gnawing blocks, 2 rabbits of F parks and 2 rabbits of M parks died or were discarded; in parks with the gnawing blocks, 3, 1, and 4 rabbits died or were discarded from F parks, M parks, and FM parks, respectively. Thus, the mortality rate was 1.7% and total losses (death + discarded animals) were 4.2%, without significant differences among the experimental groups (data not reported in tables). Neither aggressive behavior nor the presence of body lesions was observed during the trial.

The presence of gnawing blocks affected rabbit performance by increasing daily weight gain from 31 to 52 days of age (+2.0 g/day; $p<0.05$) and throughout the whole trial (+1.2 g/day; $p<0.05$), which was associated with a higher weight at slaughter (73 days) (+55 g; $p<0.05$) (Table 3.1). Feed intake and conversion were not significantly affected. The consumption of compressed hay blocks was 1.3 g/day per rabbit during the whole trial, regardless of sex-group composition (data not reported in tables).

As for sex-group composition, parks with only females and with both sexes had lower feed conversion ratios compared to parks with only males ($p<0.05$). Males also had a higher slaughter yield than females (+0.9 percentage points; $p<0.01$), whereas intermediate values were recorded in mixed-sex parks.

Table 3.1. *Effect of the presence of gnawing hay blocks and sex-group composition in collective parks on performance (31 to 72 days of age) and slaughtering results (73 days of age) of growing rabbits.*

	Gnawing hay blocks (G)		**Sex-group composition (S)**			***p*-value[1]**		**RMSE[2]**
	Absence	**Presence**	**Females**	**Males**	**Females+Males**	**G**	**S**	
Pens (n)	9	9	6	6	6			
Rabbits (n)	140	136	91	93	92			
Live weight (g)								
at 31 days	765	767	768	766	764			82
at 52 days	1936	1979	1962	1952	1958			189
at 72 days	2845	2896	2880	2850	2881			220
Live weight gain (g/d)								
31 to 52 days	55.5	57.5	56.6	56.3	56.6	*		7.6
52 to 72 days	45.5	45.9	45.9	44.9	46.2			5.9
31 to 72 days	50.6	51.8	51.4	50.7	51.5	*		4.6
Feed intake[3] (g/d)								
31 to 52 days	118	121	119	120	119			4
52 to 72 days	165	169	168	166	168			7
31 to 72 days	141	145	143	143	144			5
Feed conversion ratio[3] (g/g)								
31 to 52 days	2.23	2.20	2.20	2.24	2.20			0.08
52 to 72 days	3.64	3.68	3.65	3.69	3.64			0.12
31 to 72 days	2.87	2.87	2.86[a]	2.89[b]	2.86[a]		*	0.02
Slaughter weight (g)	2763	2818	2808	2769	2792	*		212
Carcass weight (g)	1696	1730	1712	1713	1714			147
Slaughter yield (%)	61.2	61.3	60.8[a]	61.7[b]	61.3[ab]		**	1.7

[1]Interactions between the main factors were not significant and have not been reported in Table. [2]Root mean square error. [3]At a pen level. * $p<0.05$, ** $p<0.01$. Means with different superscript letters in the same row differ significantly ($p<0.05$; Bonferroni test).

Behavioral observations

The presence of gnawing blocks did not affect the budget time of growing rabbits (Table 2), except for the time spent allo-grooming, which was higher in parks without blocks than in those with blocks (0.75% vs. 0.44% observation time; $p<0.001$) (Table 3.2). In addition, the time spent in the resting area was higher in parks without blocks (53.5% vs. 49.4%; $p<0.001$) (Table 3.2). Regarding the effect of group composition, rabbits in mixed-sex parks spent more time resting than those in single-sex parks (67.8% observation time in FM vs. 64.2% and 64.7% in F and M parks, respectively; $p<0.01$). The time spent in self-grooming was lower in the FM parks than in the F parks and M parks (12.8% vs. 15.4% and 15.8% observation time; $p<0.001$), whereas the opposite trend was observed for the time spent in allo-grooming (0.73% in FM parks vs. 0.60% and 0.46% in F and M parks, respectively; $p<0.01$). Stretching was observed more often in rabbits from FM parks than in those from F and M parks (+0.08 events per park; $p<0.05$). Finally, the time spent in the resting area was higher in parks with only females than in those with both sexes (+2.3 percentage points; $p<0.05$) (Table 3.2).

Table 3.2. *Effect of the presence of gnawing hay blocks, sex-group composition and age on behaviors (% of observation time; number of events per pen per observation period) of growing rabbits kept in collective parks across 24 h. Data are reported as means ± standard deviations.*

	Gnawing hay blocks (G)		Sex-group composition (S)			Age (A)		*p*-value[1]		
	Absence	Presence	Females	Males	Females+Males	42 d	70 d	G	S	A
Pens (n)	9	9	6	6	6	18	18			
Rabbits (n)	140	136	91	93	92	276	276			
Feeding (%)	11.8±8.0	11.2±8.3	11.8±8.3	11.8±8.4	11.2±7.8	14.1±8.7	9.1±6.7			***
Drinking (%)	1.74±2.26	1.51±2.18	1.59±2.04	1.71±2.44	1.57±2.17	1.75±2.30	1.50±2.14			**
Caecotrophy (%)	0.25±0.97	0.22±0.88	0.15±0.61	0.20±0.90	0.35±1.18	0.10±0.57	0.37±1.16			***
Sitting (%)	0.38±0.72	0.26±0.52	0.30±0.53	0.32±0.66	0.37±0.69	0.42±0.77	0.24±0.44			***
Moving (%)	0.76±0.75	0.68±0.69	0.66±0.61	0.84±0.91	0.65±0.59	0.90±0.75	0.53±0.64			***
Running (%)	0.16±0.46	0.17±0.46	0.16±0.40	0.18±0.51	0.16±0.46	0.19±0.48	0.14±0.44			
Self-grooming (%)	14.8±7.7	14.5±7.8	15.4^{b}±7.6	15.8^{b}±7.9	12.8^{a}±7.4	12.5±6.3	16.8±8.3		***	***
Allo-grooming (%)	0.75±1.50	0.44±1.05	0.60^{ab}±1.25	0.46^{a}±1.07	0.73^{b}±1.53	0.43±1.16	0.76±1.41	***	**	***
Sniffing (%)	3.50±4.32	3.08±3.73	3.80±5.00	2.92±3.61	3.16±3.25	3.29±3.86	3.28±4.21			
Biting/licking (%)	1.01±2.19	0.78±1.69	1.11±2.27	0.82±1.87	0.75±1.68	0.83±1.95	0.96±1.97			*
Total resting (%)	64.8±13.6	66.4±12.8	64.2^{a}±13.7	64.7^{a}±13.0	67.8^{b}±12.6	65.2±13.0	66.0±13.4		**	
Resting crouched body (%)	38.7±17.4	39.3±15.8	37.9±16.8	39.3±16.8	39.8±16.1	43.2±16.6	34.8±15.4			***
Resting stretched body (%)	26.0±15.6	27.1±14.5	26.3±15.6	25.3±15.0	28.0±14.5	22.0±13.2	31.1±15.4			***
Time spent in the resting area (%)	53.5±13.3	49.4±11.9	52.3^{b}±13.7	52.0^{ab}±11.8	50.0^{a}±12.9	50.7±16.1	52.1±8.2	***	*	
Resting in the resting area (%)	40.2±14.3	37.7±12.7	39.1±14.4	39.0±12.9	38.7±13.4	39.2±16.1	38.7±10.5	**		
Resting in the feeding area (%)	24.6±13.0	28.7±11.6	25.1^{a}±13.1	25.7^{a}±11.5	29.1^{b}±12.4	26.0±14.1	27.2±10.5	***	***	***
Contact with the enrichment[2] (%)	--	0.42±1.58	0.13±0.83	0.20±1.07	0.30±1.42	0.20±1.01	0.22±1.26	n.e.	n.e.	n.e.
Stretching (n)	0.19±0.47	0.22±0.47	0.19^{a}±0.43	0.17^{a}±0.44	0.26^{b}±0.53	0.23±0.49	0.19±0.45		*	
Hops (n)	0.07±0.36	0.03±0.18	0.03±0.19	0.07±0.37	0.05±0.25	0.04±0.28	0.06±0.28	n.e.	n.e.	n.e.
Rearing (n)	1.23±12.3	0.31±1.19	1.33±14.4	0.34±0.76	0.65±4.65	0.34±0.82	1.20±12.4	n.e.	n.e.	n.e.
Aggressive behaviors (n)	0.03±0.28	0.03±0.20	0.00±0.06	0.02±0.17	0.06±0.38	0.00±0.00	0.06±0.34	n.e.	n.e.	n.e.

[1]With the exceptions reported in Figures 1, 2, and 3, the interactions among the main factors were not significant and are not reported in Table. [2]Time spent sniffing, licking, or biting the compressed hay block. * *p*<0.05, ** *p*<0.01, *** *p*<0.001. n.e. = not estimable. Means with different superscript letters in the same row differ significantly (*p*<0.05; Bonferroni test).

The presence of the hay blocks affected the use of the feeding and resting areas to different extents, depending on the group composition in parks (significant interaction absence/presence blocks × sex-group composition) (Figure 3.1). In pens without blocks, the time spent in the resting area was the highest in M pens; in contrast, in pens with blocks, this time increased in the rabbits in F pens to those of M and FM pens (Figure 3.1a). Similarly, the time spent resting in the resting area decreased in the rabbits in F pens to those of M and FM pens when blocks were not included, while an opposite trend was recorded in the presence of the blocks (Figure 3.1b). In contrast, while time spent resting in the feeding area (Figure 3.1c) and time spent resting with the stretched body (Figure 3.1d) did not change with sex group composition in pens with blocks, large differences between F pens and FM pens were observed in the absence of blocks.

As age increased from 42 to 70 days, rabbits spent less time feeding and drinking (-35.2% and -14.3%, respectively; 0.01<*p*<0.001) and increased the time for cecotrophy (*p*<0.001), self- and allo-grooming (+4.3% and +0.33 percentage points, respectively; *p*<0.001), and biting/licking (+0.13 percentage points; *p*<0.05) (Table 3.2). The time spent sitting and moving decreased with increasing age. Time resting with the crouched body decreased (-19.4%), whereas time resting with the stretched body increased (+41.4%) (*p*<0.001) as age increased.

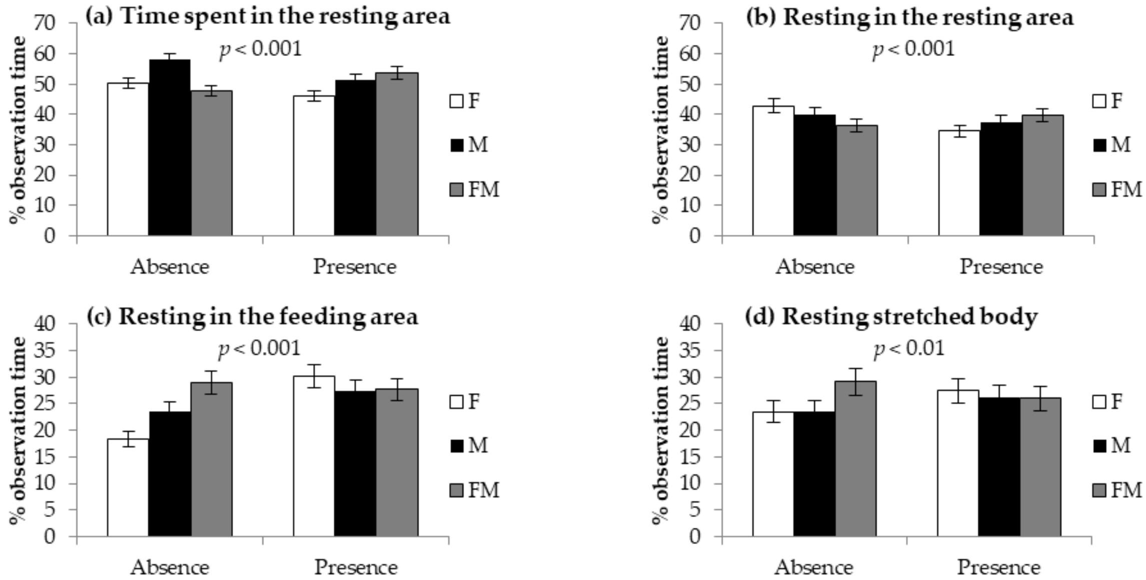

Figure 3.1. *Time (% of observation time) spent (**a**) in the resting area, (**b**) resting in the resting area, (**c**) resting in the feeding area and (**d**) resting with stretched body in growing rabbits kept in collective parks with only females (F), with only males (M) or with females and males (FM) in parks without (absence) or with (presence) gnawing hay blocks (significant interactions between group composition and presence/absence gnawing blocks). Data are reported as the means ± standard deviations.*

Significant interactions were recorded between age and the absence or presence of gnawing blocks for the use of the resting area (Figure 3.2). In fact, the time the rabbits spent in the resting area was the lowest at 42 days when the blocks were in the parks (Figure 3.2a), which coincided with the lowest time spent in the resting area (Figure 3.2b). In contrast, the lowest resting time in the feeding area was observed at 42 days for rabbits kept in parks without blocks (Figure 3.2c).

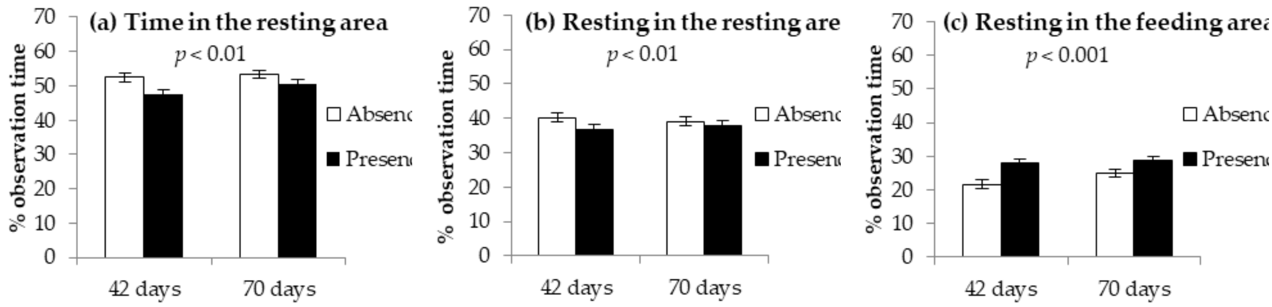

Figure 3.2. *Time (% of observation time) spent (**a**) in the resting area, (**b**) resting in the resting area, (**c**) and resting in the feeding area of the parks by growing rabbits kept in collective parks without (absence) or with (presence) gnawing hay blocks at 42 and 70 days of age (significant interactions age × absence/presence of gnawing blocks). Data are reported as means ± standard deviations.*

As for sniffing and resting behaviors, rabbits in collective parks with different sex-group compositions showed differences according to age (significant interaction age × group composition;

Figure 3.3). The lowest time spent sniffing at 42 days of age was recorded in parks with only males; whereas at 70 days of age, the lowest time was recorded in parks with both sexes (Figure 3.3a). The lowest time spent resting in the feeding area was recorded at 42 days in parks with only females; whereas the highest value was recorded in parks with mixed sexes at 42 and 70 days (Figure 3.3b). While no difference was observed in the time spent resting with the crouched body according to group composition at 42 days, at 70 days the lowest value was recorded for rabbits in F parks (Figure 3.3c). Finally, the time spent resting with the stretched body was lowest in single-sex parks at 42 days and highest in F parks at 70 days of age (Figure 3.3d).

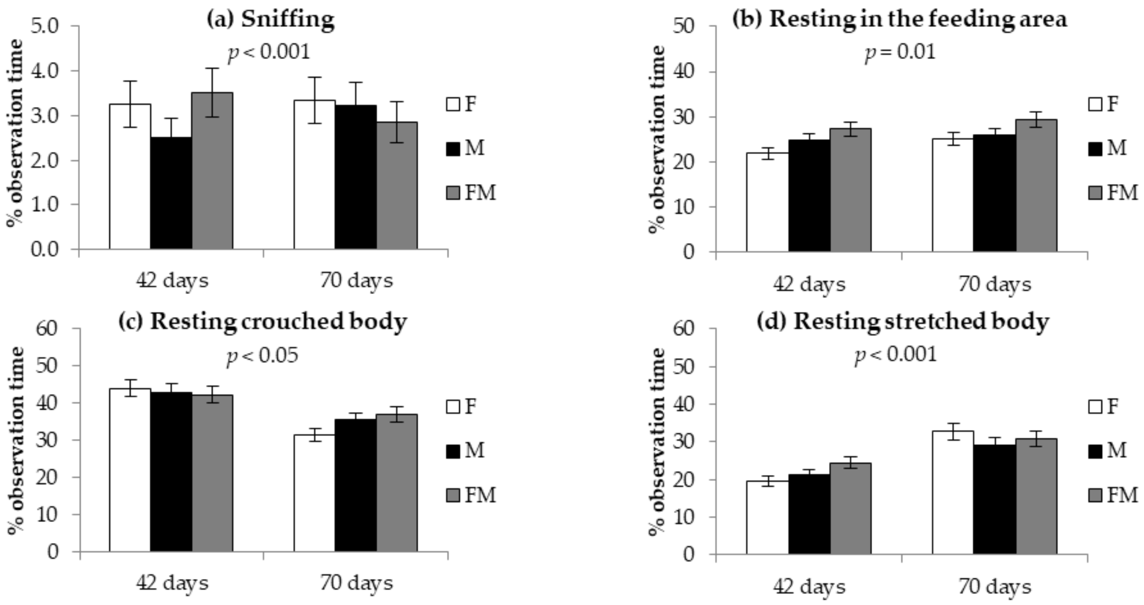

Figure 3.3. *Time (% of observation time) spent (**a**) sniffing, (**b**) resting in the feeding area of the park, (**c**) resting with crouched body, and (**d**) resting with stretched body in growing rabbits kept in collective parks with only females (F), only males (M) or with males and females (FM) at 42 and 70 days of age (significant interactions between sex-group composition and age). Data are reported as means ± standard deviations.*

Reactivity tests

The rabbits that were provided with gnawing blocks spent more time moving during the open field test (+9.9%; $p<0.05$; Table 3.3) and approached the bottle earlier during the novel object test (61.6% vs. 49.3%; $p<0.05$; Table 3.4) compared to rabbits that were kept without blocks. Nevertheless, after five minutes of testing, no difference in the novel-object test or human approach test was recorded between the rabbits of the two treatments (Table 3.4).

As for sex-group composition, during the open field test, rabbits in parks with both sexes spent more time moving (+22.5% vs. rabbits of F and M parks; $p<0.05$), while rabbits in parks with only males displayed self-grooming for a longer time (+49.2% vs. rabbits of F and FM parks; $p<0.01$)

(Table 3.3). In the human approach test, some differences were recorded during the last minute of the test; however, there were no marked differences in reactivity towards men among rabbits kept in parks with different sex-group compositions (Table 3.4).

Table 3.3. *Effect of the presence of gnawing hay blocks and sex-group composition age on behaviors of growing rabbits kept in collective parks during the open-field test at 64 days of age. Data are reported as the means ± standard deviations.*

	Gnawing hay blocks (G)		**Sex-group composition (S)**			***p*-value[1]**	
	Absence	**Presence**	**Females**	**Males**	**Females+Males**	**G**	**S**
Rabbits (n)	54	54	36	36	36		
Entered animals (%)	59.3±56.6	57.4±60.3	52.8±61.2	61.1±53.4	61.1±57.0		
Latency (s)	19.3±12.2	26.4±15.5	15.9±11.0	26.4±14.4	25.0±15.2		
Total displacements (n)	32.6±24.8	32.8±25.3	30.2±23.8	31.8±24.1	36.1±27.1		
Central displacements (s)	2.19±3.22	1.63±2.82	1.89±3.23	1.39±2.09	2.44±3.56		
Exploration (s)	366±67	365±50	360±53	368±71	369±53		
Movement (s)	29.4±20.8	32.3±22.9	28.5[a]±21.1	28.8[a]±18.9	35.1[b]±25.0	*	*
Running (s)	2.20±4.17	1.37±3.89	1.56±3.22	2.28±5.32	1.53±3.28		
Standing still (s)	55.6±45.9	67.1±52.6	70.1±45.9	57.9±50.6	56.1±51.9		
Self-grooming (s)	3.28±4.47	3.96±6.36	2.94[a]±4.49	4.64[b]±7.08	3.28[a]±4.47		**
Escape attempts (n)	0.16±0.45	0.26±0.77	0.05±0.23	0.09±0.29	0.45±0.96	n.e.	n.e.
Resting (s)	17.5±52.7	7.57±24.5	13.4±37.7	14.7±50.3	9.53±30.0	n.e.	n.e.
Biting (s)	5.54±1.27	2.41±6.66	2.92±8.41	3.86±10.22	5.14±11.83	n.e.	n.e.
Digging, (s)	0.41±2.34	0.15±0.66	0.19±0.75	0.11±0.46	0.53±2.84	n.e.	n.e.
Urination (n)	0.02±0.14	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.17	n.e.	n.e.
Rearing (n)	1.24±2.91	0.91±2.54	1.11±2.82	0.30±0.75	1.80±3.62	n.e.	n.e.
Hops (n)	0.02±0.14	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.17	n.e.	n.e.

[1] Interactions between the main factors were not significant and not reported in Table. * $p<0.05$, ** $p<0.01$. n.e. = not estimable. Means with different superscript letters in the same row differ significantly ($p<0.05$; Bonferroni test).

Table 3.4. *Effect of the presence of gnawing hay blocks and sex-group composition on the contacts rabbits-object (rabbits that touched the object, % present rabbits) during the novel-object test at 65 days of age and on the contacts rabbits-man (rabbits that touched the man, % present rabbits) during the human-approach test at 67 days in growing rabbits kept in collective parks. Data are reported as the means ± standard deviations.*

	Gnawing hay blocks (G)		**Sex-group composition (S)**			***p*-value[1]**	
	Absence	**Presence**	**Females**	**Males**	**Females+Males**	**G**	**S**
Pens (n)	9	9	6	6	6		
Rabbits (n)	140	136	91	93	92		
Novel-object test							
Rabbits-object contacts (%)							
1-60 s	49.3±49.7	61.6±50.1	57.9±49.6	62.1±49.2	45.6±50.8	*	
61-180 s	22.9±42.6	13.0±32.7	14.7±36.2	16.8±37.6	22.8±45.1	*	
181-300 s	9.03±74.6	5.08±31.2	6.32±21.9	6.32±86.8	9.80±30.7		
1-300 s	81.2±39.0	80.4±40.8	78.9±41.0	85.3±34.7	78.3±42.2		
Human-approach test							
Rabbits-human contacts (%)							
1-60 s	30.6±46.1	34.5±49.0	35.8±49.6	28.4±45.3	33.3±47.0		
61-180 s	8.3±51.8	7.9±71.1	3.2±51.0	10.5±30.9	10.7±30.7		
181-300 s	6.3±59.2	11.5±77.0	3.2^{a}±48.0	13.7^{b}±34.7	9.7^{b}±27.8		*
1-300 s	45.1±52.1	54.0±51.3	42.1±52.5	52.6±50.1	53.7±50.8		

[1]Interactions between the main factors were not significant and are not reported in Table * p<0.05. Means with different superscript letters in the same row differ significantly (p<0.05; Bonferroni test).

DISCUSSION

Under the conditions of conventional rearing systems, growing rabbits have traditionally been kept in barren enclosures with little space for movement and few possibilities of fully expressing their species-specific behaviors, such as gnawing and social interactions. Many of the behavioral needs of rabbits under farming conditions are not known, nor have the effects of their restrictions been assessed (EFSA, 2020). Thus, the present study was intended to contribute to improving knowledge about rabbits' behavioral needs and to the development and optimization of alternative cage-free housing systems for rabbits.

Effect of gnawing blocks

Among other behaviors, whether it is recognized that rabbits under wild conditions have the opportunity of gnawing besides foraging, the presence of gnawing objects under farming conditions has been claimed to improve their welfare by increasing locomotion (Postollec et al., 2002; Maertens et al., 2013), reducing stress (Buijs et al., 2011), and reducing negative social interactions in collective housing systems (Princz et al., 2008). On the other hand, based on available literature, most studies reported that the provision of gnawing materials does not affect the productive performance of individually or pen-housed growing rabbits (Princz et al., 2007; Maertens et al., 2013; Bozicovich et al., 2016), which was confirmed under the conditions of the present study.

The preference and time spent interacting with different gnawing objects can vary according to their physical and nutritional characteristics (Princz et al., 2007; Bozicovich et al., 2016). Wooden boards (Buijs et al., 2011), cardboard and rubber chewing materials (Poggiagliolmi et al., 2011; Gomes da Silva et al., 2021), straw (Postollec et al., 2002), and gnawing blocks (Maertens et al., 2013; Gomes da Silva et al., 2021) have been tested. When comparing wooden sticks, soft-wood sticks were preferred over hard-wood sticks (Princz et al., 2007; Bozicovich et al., 2016). Indeed, the compressed hay blocks used in the present trial were expected to be rather soft to gnaw, and rabbits were found to have an average observation time of 0.4% (sniffing, licking, or gnawing). We hypothesize that interest in such objects could have been higher in the case of individuals than in collective housing. Nevertheless, even growing rabbits kept in individual cages spent little time, 0.01-0.21% observation time) in gnawing wooden sticks (Jordan et al., 2008). If considered simply as enrichments, one could argue that the low interaction time with the gnawing blocks depended on the reduction of the novelty effect over time (Johnson et al., 2003) because they were continuously available. The position of the objects in the enclosures (on the floor, rather than on the ceiling or on a wall) could also modify their use, despite the implications for feces and bacterial contamination (Morisse et al., 1999).

Under our conditions, despite the scarce effects on the budget time and the little time in absolute value addressed to the gnawing blocks, their presence affected the use of the different areas of the park and the animal distributions, in addition to animal reactivity towards a new environment in the open field test and in the novel object test that are worthy of consideration.

As for the use of the different areas of the park, in the present trial rabbits spent 51.5% observation time in the resting area; they rested 39.0% of observation time in the resting area and 26.7% observation time in the feeding area (averages of the two observation days). Morisse et al., 1999 also showed that rabbits prefer to rest in the park aside from the feeders, likely because of less disturbance due to the animals' access to feeders. Based on this preference, we also provided the resting area with a plastic floor, which has been proven to be preferred over a wire net by rabbits (Matics et al., 2003; Alfonso-Carrillo et al., 2014 a, b). Nevertheless, the presence of blocks in the feeding area contributed to the presence of rabbits in this area compared to parks without blocks, which was likely due to the animals' interest in this provision. In fact, when they were free to move among the four cages through swing doors, growing rabbits preferred cages provided with gnawing sticks (Princz et al., 2008). In our study, this effect was confirmed over time (both at 42 and 70 days) and with different sex-group compositions.

Regarding behavior, previous studies performed on rabbits kept in cages in small groups (2-4 or 6) reported increased allo-grooming and social interactions (Zucca et al., 2012; Bozicovich et al., 2016) and decreased self-grooming with the provision of gnawing sticks, which was attributed to higher pheromonal olfactory stimulation and mutual recognition (Zucca et al., 2012). In contrast, in the parks used in the present study, in which social interactions were obviously higher than in cages, allo-grooming decreased in the pens with gnawing blocks. Gnawing sticks, as well as other environmental enrichments, have been found to reduce the incidence of abnormal behaviors in cages (e.g., stereotypies with cage manipulation) (Luzi et al., 2003; Jordan et al., 2004) and aggression among animals in collective systems (Princz et al., 2007, 2008). In cages with six rabbits, Bozicovich et al. (2016) reported increased aggressive behavior, but a lower occurrence of injured rabbits in enriched cages than in non-enriched ones. Under the conditions of our study, no evidence of this mitigation effect was found because of limited aggression and the absence of injured animals at the end of the trial, regardless of the treatment. The relatively early age at slaughter (73 days) and moderate group size (16 animals per group) could have accounted for this good result. In fact, aggressive interactions increase as animals' approach sexual maturity, and injury occurrence has been reported in rabbits slaughtered later (15.0% and 22.0% at 76 and 83 days) (Trocino et al., 2015) or kept in larger groups (15.6% at 68 days and 18.0% in groups with 27 and 36 rabbits at 75 days) (Trocino et al., 2019a) than in the present trial.

Finally, the results from the reactivity tests performed in the current study also indicate a positive effect of the provision of gnawing blocks, where a higher movement activity during the open field test and a quicker approach to the novel object during the test can be considered as active and positive reactions to new stimuli (Trocino et al., 2013, 2015, 2019a). On the other hand, the absence of differences in reactivity in the human approach test confirms previous observations about the positive effect of collective housing/conspecific presence in reducing fear levels towards men compared to rabbits housed individually. In fact, no difference in the reaction towards humans in the tonic immobility test was reported even for rabbits kept in bicellular cages compared to those kept in small (Trocino et al., 2013) or large groups (Trocino et al., 2014, 2019a).

Effect of sex-group composition and interaction among experimental factors

In view of growth performance, the present study confirmed that there is no need for single-sex rearing of growing rabbits, despite some differences in growth performance (Di Meo et al., 2003; Trocino et al., 2015; Birolo et al., 2020) or slaughter yield (Petracci et al., 1999; Trocino et al., 2003, 2015).

In contrast, under our conditions, the budget time varied according to group composition. Increased resting time and to a lesser extent, social interactions (allo-grooming) and decreased self-grooming, were observed in mixed-sex groups compared to single-sex groups. Similarly, a higher occurrence of social interactions and a lower occurrence of stereotypes were recorded by Bozicovich et al. (2016) in rabbits kept in mixed-sex groups in cages of six compared to single-sex groups, whereas other authors did not report differences in behavior according to sex-group composition in cages (Szendrő et al., 2012). Whether the above-mentioned differences in behavior can be interpreted as improved welfare for rabbits reared in mixed-sex groups is not definitively stated based on observations of injured animals. In fact, a higher rate of wounds was found in mixed-sex parks than in single-sex parks by Di Meo et al. (2003) and in only male or mixed-sex parks than in only female parks (Birolo et al., 2020) or cages (Bozicovich et al., 2016). In addition, in mixed-sex parks, the rate of injured rabbits was noticeably higher among males than females (25.8% vs. 11.3%; $p \leq 0.001$) at the end of the fattening period (Trocino et al., 2015). Thus, aggression in mixed-sex parks is likely due to males, which is consistent with evidence that aggression is more severe among males than females, especially when sexual maturity approaches and rabbits are reared in large groups (Bigler and Oester, 1996; Verga et al., 2007).

In accordance with other studies performed under different housing conditions (Morisse et al., 1997; Dal Bosco et al., 2002; Buijs et al., 2011; Trocino et al., 2019a), on average of the two observation days, our rabbits spent most of their time resting, self-grooming, feeding, sniffing, and drinking. As for the differences between 40 and 72 days of age, the greatest changes were due to the reduction in feeding time, as observed by other authors (Morisse et al., 1999; Martrenchar et al., 2001; Trocino et al., 2013, 2019a), and the increase in self-grooming. Differently, in larger parks (1.68 m^2 vs. 1.00 m^2 of the present trial) and with larger group size (20 and 27 animals per park), as age increased from 56 days to slaughtering age (76 and 83 days of age), the greatest change was the increased resting time, especially with a crouched body, at the expense of comfort behaviors (self- and allo-grooming) and exploration (sniffing, moving, running). Differences in the architecture of the park systems in the two studies (unique free area in Trocino et al. (2019a) and two connected modules in the present study) could also account for the different uses of space and time.

The use of the areas changed in parks with different sex-group compositions depending on the absence/presence of gnawing blocks, even if it is difficult to understand the reasons and welfare consequences for the observed differences.

As for the time the animals spent in the resting and feeding areas, the interactions we observed between rabbit age and the provision of gnawing blocks were likely related to the increase in animal size. On average, the rabbits were homogenously distributed between the resting (50% total observation time) and feeding areas (50%). At both ages, however, the time spent in the resting area was lower in parks with gnawing sticks than in parks without, but differences decreased with age (from -5.0 percentage units at 42 days to -2.9 percentage units at 70 days), likely because of the increased stocking density (kg live weight/m^2) and the need for a comfortable spatial distribution in the two areas of the park.

CONCLUSIONS

Regarding knowledge about the rabbits' behavioral needs, the changes in the rabbits' distribution in the park proved the interest of the animals towards the provision of the blocks, which is consistent with the satisfaction of the animal's behavioral needs. Although the rabbits spent a rather short time interacting with the compressed blocks used in the present study and showed few changes in the overall budget time, they showed an active and positive response towards a new environment and a new object when provided with the gnawing blocks, which can stand for reduced stress and improved welfare. Thus, the overall results related to production, behavior, and reactivity represent the provision of gnawing blocks in collective parks for welfare improvement, while they do not support the change from current mixed-sex rearing to single-sex. Finally, under the conditions of our study, no conclusion can be drawn regarding the effects on aggressive interactions, as no problem was recorded in this regard.

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4. Contribution 2: A pilot study about on-farm assessment of health and welfare in rabbits kept in different housing systems

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INTRODUCTION

The Farm to Fork strategy (European Commission, 2020) of the European Green Deal (European Commission, 2019) calls for new and revised regulations for the protection of the welfare and health of farmed animals. As for rabbits, during the last decades, consumers' concerns about farming practices and housing systems have grown (Council of Europe, 1998; Trocino et al., 2019). The European Parliament Resolution (2017) on minimum standards for the protection of farmed rabbits called for alternatives, which were definitively stated by the European Parliament Resolution on the European Citizens' Initiative End the Cage Age (European Parliament, 2021), asking the Commission to phase out cages in all European farms, possibly by 2027, for any farmed animals.

In Europe, commercial farms of meat rabbits are mostly located in Spain, France, and Italy, which account for 83% of European production (Tubiello et al., 2013; European Commission, 2017), while in many other countries, rabbits are popular only as pets. Farming of meat rabbit shows a wide variability both among and within countries (Trocino et al., 2006; Italian Ministry of Health, 2019). The majority of commercial farms use cages, i.e., standard breeding cages for reproducing does with their litters associated with bicellular cages for growing rabbits, or dual-purpose cages for both reproducing does with their litters and growing rabbits. Some farms use structurally enriched cages (EFSA, 2020), whereas few commercial farms use alternative systems based on parks (also called elevated pens) that can house growing rabbits for fattening in large groups (usually 30-35) and reproducing does with their litters in individual systems or, seldom, in continuous or part-time groups (Szendrő et al., 2019; EFSA, 2020). Park housing systems have been tested during the last decades but are not yet widespread or validated at a commercial level all over Europe, for which the technical standards for their implementation are not yet fully available (European Parliament, 2017). Park systems have also shown some weaknesses in terms of health and welfare of growing rabbits due to aggression and to diseases transmitted through the oro-fecal route (European Commission, 2017; European Parliament, 2017), besides being associated with elevated levels of aggression and stress when reproducing does are reared in groups (Szendrő et al., 2019; EFSA, 2020). From the perspective of cage phasing-out, these issues generate deep uncertainty in farmers and technicians, as the rabbit sector has also been hit hard with the decline in meat consumption and the economic crisis. Sales prices have fallen by ~ 20% in 3 years, while production costs are significantly and continuously increasing (European Parliament, 2017). There is only one study published about the economic performances of rabbit farms. It shows that enriched cages are economically sustainable and comparable to conventional housing systems with bicellular or dual-purpose cages and provide a significant reduction in drug use in the tested farms (Mondin et al., 2021). At the same time, no information is available about farmer perception and willingness to change which could be driving factors for adapting production systems to rabbit welfare needs.

The latest Scientific Opinion of the European Food and Safety Authority (EFSA) (2020) compared the health and welfare of rabbits kept in different housing systems by a global impact score, based on both health- and behavior-related welfare consequences, obtained through an Expert Knowledge Elicitation (EKE) process. The probabilistic analyses of EKE results showed that cage systems are likely associated with lower rabbit welfare, mainly because of behavioral restrictions and concerns. However, field data about the prevalence of welfare consequences are missing. Moreover, unlike other species, no validated animal-based measures (ABMs) or protocols to assess on-farm animal welfare are available for rabbits yet (Blokhuis et al., 2010). Some measures and protocols have recently been tested only on farms using standard barren cages (Bignon et al., 2017; Dalmau et al., 2020). Therefore, this pilot study aimed to provide a preliminary evaluation of on-farm health and welfare of rabbits kept in different housing systems based on a protocol using resource-, management-, and animal-based measures, along with including a number of the few commercial farms that were using alternative systems. In detail, the protocol was tested in the following housing systems: (1) the standard cage system, using standard breeding cages for reproducing does with their litters associated with bicellular cages for growing rabbits; (2) the dual-purpose cage system, where dual-purpose cages are used for both reproducing does with their litters and growing rabbits; (3) the enriched cage system, based on enriched cages for both reproducing does with their litters and growing rabbits; and (4) the park system, which uses single modules of a park for reproducing does with their litters and four joined modules as a larger park for growing rabbits.

MATERIALS AND METHODS

Farms and housing systems

A total of 12 commercial farms located in the Northeast of Italy took part in the assessments. All farms were closed cycle, with a population size between 456 and 3890 reproducing does.

These farms were proposed by practitioners working in the field, based on the farmers' availability, to have a sample of three farms per housing system, i.e., (1) the standard cage system; (2) the dual-purpose cage system; (3) the enriched cage system; and (4) the park system.

In the case of the standard cage system, at weaning, the litters were moved from the breeding cages to the bicellular cages, while the reproducing does always remain in the original cages. In these farms, the size of the standard breeding cage was 3300 cm^2, whereas the size of the bicellular cage was 1200 cm^2 (Table 1).

In the farms with the other housing systems, dual-purpose cages were smaller (3655 cm^2) than enriched cages (4739 cm^2). Enriched cages were equipped with a wire-mesh elevated platform (1015 cm^2). Parks (30,977 cm^2) were made up of four modules (each 7744 cm^2) joined by removing the wire net walls between them (Table 1). Parks had a plastic-mesh platform (2282 cm^2 for a single module and 9129 cm^2 for a park) and a plastic-slatted floor (Supplementary Figure 1).

In farms using the dual-purpose cage, enriched cage, and park systems, at weaning, the does were moved to clean cages or to clean individual modules of the parks, while the litters remained where they were born until slaughtering. In the farms using parks, at weaning, four contiguous modules were joined by removing wire walls between them to obtain parks in which growing rabbits from four/five litters were kept until slaughtering in large groups (32-40 rabbits).

As detailed in Supplementary Table 1, besides the housing system, farms differed in several other factors, such as animal genotype (Hyla, Grimaud, or Martini commercial crossbreed), reproduction rhythm (does artificially inseminated at 11 days or 18 days after kindling), building type (indoor or semi-plein air), ventilation system (extraction with/without cooling system), and the presence of plastic mats in the cages, diets, and feeding programs for growing rabbits (ad libitum or restricted). The weaning age of litters ranged from 32 to 38 days and the slaughtering age of growing rabbits ranged from 71 to 86 days, due to market requirements, besides the farm's own organization. Within the different housing systems, it should be noted that (1) only one farm using enriched cages adopted the genotype Martini; (2) farms using enriched cages adopted only the reproduction rhythm with insemination 11 days after kindling; (3) farms with standard cages did not use foot mats in cages for reproducing does; (4) farms with enriched cages and parks only used ad libitum feeding for growing rabbits, while, in farms with standard and dual-purpose cages, both feeding systems were used. These issues have been considered in the discussion of results.

Table 1. *Housing systems and cage size in the farms subjected to the on-farm welfare evaluation in reproducing does with their litters and in growing rabbits.*

Housing system	**Standard cage system**		**Dual-purpose cage system**	**Enriched cage system**	**Park system**	
Type of cage	**Breeding cage**	**Bicellular cage**			**Single module**	**Park (four modules)**
Rabbit category	Reproducing does with their litters	Growing rabbits	Growing rabbits; reproducing does with their litters	Growing rabbits; reproducing does with their litters	Reproducing does with their litters	Growing rabbits
Total available surface (cm^2) *	3300	1200 (1008-1584)	3655 (3315-3927)	4739 (4522–5082)	-	30,977 (30,814–31,304)
Available surface/rabbit (cm^2)	3300	600	609	592	-	860
Growing rabbits (n/cage)	-	2	6	8	-	36 (32–40)
Growing rabbits (n/m^2)	-	17 (13-20)	16 (15-18)	17 (16-18)	-	12 (10–13)
Live weight at slaughtering (kg/m^2)	-	46.0 (33-56)	44.0 (40-49)	44.1 (39-47)	-	30.1 (29–32)

*Including the nest area and the platform surface when available

On-farm recordings and sampling

Recordings were scheduled to cover three seasons (i.e., autumn, winter, and summer) with two visits per season per farm: (1) a pre-weaning visit, the week before weaning (27-31 days after kindling) for recordings on reproducing does and litters, and (2) a pre-slaughtering visit, 2-5 days before slaughtering for recordings on the corresponding growing rabbits.

Resource and management-based indicators besides ABMs were recorded in does with their litters on the pre-weaning visit (Table 2) and in growing rabbits on the pre-slaughtering visit (Table 3). On each visit, farm temperature and humidity were measured using an Anemometer Kestrel 5000 (Nielsen-Kellerman Company, Boothwyn, PA, USA); NH_3 and CO_2 gases were recorded by a Gas Detector X-am 7000 (Draeger, Lübeck, Germany).

At every pre-weaning visit, for a random sample of 75 does (12-15 at their first kindling), the does' body weight, body condition score (BCS), and health status were individually evaluated (Table 2). The BCS was assessed by palpating the fullness of muscle and fat in the lumbar and gluteal regions based on a five-point scale (0-5) (Bonanno et al., 2005). Symptoms related to respiratory (nasal and/or ocular secretion) and digestive (diarrhea) problems, mastitis, ulcerative pododermatitis, and dermatomycosis were also scored. The litter size and weight and the kit health (symptoms of respiratory and digestive problems, dermatomycosis) were also assessed (Table 2).

During the pre-slaughtering visits, body weight, signs of diarrhea, and lesions related to aggression and dermatomycosis were individually assessed on a random sample of 100 growing rabbits per visit (2 rabbits each × 50 bicellular cages, dual-purpose cage, and enriched cages; 20 rabbits × 5 parks) (Table 3).

By the end of the trial, out of the initially selected 12 farms, one farm with a dual-purpose cage system was available only for two seasons (i.e., two pre-weaning and two pre-slaughtering visits in autumn and winter) and one farm with parks was available only for one season (i.e., one pre-weaning visit and one pre-slaughtering visit in autumn). Health data of growing rabbits were not recorded in autumn because of the unavailability of some farmers.

Recordings ran from September 2018 to August 2019. The pre-weaning visits lasted on average 90 min, while the pre-slaughtering visits took 60 min. Both types of visits involved two assessors.

In the autumn and summer seasons, while weighing, hair samples were collected from 10 animals at random per visit from both reproducing does at the pre-weaning visits and from growing rabbits at the pre-slaughtering visits. Hair was gently collected using a brush from rabbits' back region and hind legs, individually packed in plastic bags, and soon transferred to the labs of the University of Padova, where they were stored at −20°C until analysis for cortisol.

Table 2. *Health and behavioral animal- based indicators and resource and management-based data recorded on farm in reproducing does and kits the week before weaning (pre-weaning visit).*

<table>
<tr><th>Sample size</th><th>Indicator type</th><th>Indicators</th><th>Scores</th></tr>
<tr><td rowspan="21">75 animals /farm/visit</td><td rowspan="3">Resource-based</td><td>Cage or park system</td><td>Standard cage / dual-purpose cage / enriched cage / park</td></tr>
<tr><td>Cage characteristics</td><td>Footrest presence/absence</td></tr>
<tr><td>Temperature, relative humidity, NH_3 and CO_2 concentrations</td><td>Measurements at 5 locations in the barn (4 lateral and 1 central)</td></tr>
<tr><td rowspan="3">Management-based</td><td>Animal genotype</td><td>-</td></tr>
<tr><td>Reproductive rhythm</td><td>11 d after kindling /18 d after kindling</td></tr>
<tr><td>Weaning age</td><td>-</td></tr>
<tr><td rowspan="15">Animal-based</td><td>Doe physiological status</td><td>Primiparous/pluriparous; pregnant (yes/no)</td></tr>
<tr><td>Doe body weight</td><td>-</td></tr>
<tr><td>Doe body condition score</td><td>Five-point scale (0-4; 0: cachexia; 4: obesity) (Bonanno et al., 2005)</td></tr>
<tr><td>Doe health concerns</td><td></td></tr>
<tr><td>Respiratory symptoms</td><td>Nasal and/or ocular secretion (yes/no)</td></tr>
<tr><td>Diarrhoea</td><td rowspan="2">yes/no</td></tr>
<tr><td>Mastitis</td></tr>
<tr><td>Ulcerative pododermatitis</td><td rowspan="2">yes/no and severity (1: minor and limited lesions; 2: extended lesions; 3: deeper, extended, and open lesions)</td></tr>
<tr><td>Dermatomycosis</td></tr>
<tr><td>Litter weight</td><td></td></tr>
<tr><td>Litter size</td><td></td></tr>
<tr><td>Kit health concerns</td><td></td></tr>
<tr><td>Respiratory symptoms</td><td>Nasal and/or ocular secretion (yes/no)</td></tr>
<tr><td>Diarrhoea</td><td>yes/no</td></tr>
<tr><td>Dermatomycosis</td><td>yes/no</td></tr>
<tr><td></td><td></td><td>Kit mortality</td><td>Average data at farm level</td></tr>
</table>

Table 3. *Health and behavioral animal- based indicators and resource and management-based data recorded on farm in growing rabbits before slaughtering (pre-slaughtering visit).*

Sample size	Indicator type	Indicators	Scores
100 animals /farm/visit	Resource-based	Cage or park system	Standard cage / dual-purpose cage / enriched cage / park
		Cage characteristics	Available surface (cm^2)
		Stocking density	Animals/cage, animals/m^2, kg/m^2
		Temperature, relative humidity, NH_3 and CO_2 concentrations	Measurements at 5 locations in the barn (4 lateral and 1 central)
	Management-based	Animal genotype	-
		Feeding system	Ad libitum / restricted
		Slaughtering age	
	Animal-based	Body weight	
		Health concerns	
		Respiratory symptoms	Nasal and/or ocular secretion (yes/no)
		Diarrhoea	yes/no
		Injuries associated to aggression	yes/no and severity
		Mortality	Average data at farm level

Hair cortisol analysis

Hair samples (50 mg) were homogenized in a mortar with pestle and liquid nitrogen, mixed with 5 ml of absolute methanol, and placed at 50°C in an oven for 18 h. Next, the tubes were centrifuged for 15 min and the supernatant was brought to dryness in a nitrogen stream. The dry extract was recovered with phosphate buffer and loaded onto a microplate for the cortisol assay. The antibody anti-cortisol used (Analytical Antibodies, Bologna, Italy) had the following cross-reactivities: cortisol 100%, prednisolone 44.3%, 11-deoxycortisol 13.9%, cortisone 4.95%, corticosterone 3.5%, prednisone 2.7%, 17-hydroxyprogesterone 1.0%, 11-deoxycorticosterone 0.3%, dexamethasone 0.1%, progesterone <0.01%, 17-hydroxypregnenolone <0.01%, and pregnenolone <0.01). At the validation tests, the regression curve between the steroid concentration and the reciprocal of the dilution factor showed good parallelism ($y = 19.3x - 0.2$; $R^2 = 0.999$); optimal results were also obtained for repeatability (intra-assay CV = 3.6%) and extraction yield (76%).

Statistical analysis

All data were analyzed using SAS 9.4 software (SAS, 2013). Performance data of does, litters, and growing rabbits were given as input to an ANOVA using the MIXED procedure and by fitting the linear mixed model with housing system (standard cage; dual-purpose cage; enriched cage; park), season (autumn, winter, and summer), and their interaction as fixed effects and the farm as a random effect to account for the specificity of each farm with all the different production factors within a farm. The structure variance components were used to model variance and covariance matrices.

Data related to the prevalence of health concerns were first coded as binary variables (YES/NO). Then, the average prevalence per farm and per cycle was calculated and data were given as the percentage of animals affected by a health concern with respect to the total number of animals assessed per visit per farm. Prevalence data were analyzed with the GLIMMIX procedure of SAS with a model considering housing system, season, and their interaction as the main effects. A Poisson distribution was assumed for these data.

Then, to explore the possible effects of the different production factors besides the housing system, a risk factor analysis (Rosell and de la Fuente, 2008, 2018) for performance data was carried out using the GLM procedure of SAS and by fitting a model with housing system, season, animal genotype, reproductive rhythm, parity order, and footrest presence for reproducing does and feeding system (restriction or not) for growing rabbits. For health prevalence data, the same model was fitted with the GLIMMIX procedure, assuming a Poisson distribution for these data.

Lastly, hair cortisol contents of reproducing does and growing rabbits were analyzed using the MIXED procedure and by fitting a model with housing system, season (autumn and summer), and their interaction as fixed effects and the farm as a random effect. The structure variance components were used to model variance and covariance matrices.

RESULTS

Pre-weaning visit

At the first visit, average temperatures were rather similar among farms using different housing systems (Table 4). The lowest minimum value (12.5°C) was recorded in farms with the standard cage system, whereas the maximum temperature ranged from 24.7°C in farms with the park system to 28.5°C in farms with the standard cage and enriched cage systems. The average relative humidity values were similar among farms (64.0-67.6%) (Table 4). The highest levels of CO_2 and, especially, ammonia was recorded in farms with the standard cage and dual-purpose cage housing systems. Ranges of variations from minimum to maximum values for air gases were quite large within and among housing systems.

As for ABMs (Table 4), the reproducing does in the farms with the standard cages showed the lowest live weight (4431 vs 4765 g vs 4914 and 4968 g; p<0.001) compared to the does in the farms using the dual-purpose cage and, especially, enriched cage and park systems, while BCS was the lowest in does kept in farms with standard cages compared to those kept in farms with enriched cages and parks (1.91, 1.94, 2.00, and 2.09; p<0.001). As for litter size, the lowest values were found in the farms using the standard cage and dual-purpose cage systems compared to those using the enriched cage and park systems (8.08 and 8.21 vs 8.61 and 9.18; p<0.001). The prevalence of health concerns did not differ among does or their litters kept on farms with different housing systems (Table 4). The average prevalence of diarrhea in the does ranged from 5.2 to 7.0%, pododermatitis lesions ranged from 0.0 to 7.9%, mastitis ranged from 0.0 to 6.1%, dermatomycosis ranged from 2.7 to 3.2%, and respiratory symptoms ranged from 0.0 to 0.6% without significant differences among housing systems (Table 4).

As for the effect of season, the does were heavier in autumn and lighter in summer (4841 vs 4566 g) with intermediate values in winter (4775 g; p<0.001). The kits were lighter in summer as well and heavier in winter than in autumn (588 vs 616 vs 641 g p<0.001). In contrast, no influence of the season on health issues was observed, except for diarrhea in does, which had a higher prevalence in autumn and summer than in winter (7.7% and 6.7 vs 3.5%; p<0.01) (Table 4).

The analysis of the risk factors for the performance of reproducing does and kits confirmed the significant effects of the housing system and season, besides genotype, reproductive rhythm, doe parity order, and footrest presence (Table 5; Supplementary Table 2). Parity order was a risk factor for pododermatitis and dermatomycoses, whereas footrest presence played a role in pododermatitis occurrence.

Hair cortisol level in reproducing does was lower in the farms using the standard cage and park systems (1.17 ng/g) than in those using the dual-purpose cage and enriched cage housing systems (1.57 and 1.60 ng/g; $p<0.01$) (Figure 1A) and on samples collected in autumn compared to those collected in summer (1.12 vs 1.64 ng/g $p<0.001$) (Figure 1B).

Table 4. *Results of the pre-weaning visit in farms with different housing systems across three seasons: environmental (means and intervals) and animal-based measures (means) in reproducing does and kits.*

	Housing system					**Season**				**RMSE**
	Standard	**Dual-purpose**	**Enriched**	**Park**	**P-value**	**Autumn**	**Winter**	**Summer**	**P-value**	
Environmental data										
Recordings (no)	9	8	9	7		12	11	10		
Temperature (°C)	21.3 (12.5-28.6)	20.1 (14.3-26.6)	21.7 (17.0-28.5)	20.1 (14.4-24.7)		21.1 (18.9-24.9)	15.7 (12.5-18.4)	26.5 (24.6-28.6)		
Relative humidity (%)	67.6 (55.7-79.4)	65.5 (54.0-76.7)	63.9 (35.2-79.4)	64.0 (55.1-77.6)		66.4 (55.7-79.4)	58.4 (35.2-71.8)	72.2 (55.1-79.4)		
CO_2 (ppm)	1042 (500-1914)	1260 (480-1880)	986 (100-1740)	1000 (540-1420)		1103 (100-1914)	1707 (1420-1880)	656 (480-1280)		
NH_3 (ppm)	9.9 (0.0-31.4)	10.7 (2.8-21.2)	4.6 (1.0-7.2)	6.3 (2.0-9.6)		9.0 (0.0-31.4)	12.9 (6.6-17.6)	3.9 (1.8-7.2)		
Kit mortality (%)	5.3 (0.0-14.0)	5.7 (3.0-9.0)	5.0 (0.0-15.0)	5.0 (3.0-8.0)		3.0 (0.0-5.0)	7.6 (3.0-15.0)	5.3 (2.0-14.0)		
Animal based measures										
Does and litters (no.)	675	625	700	300		825	750	675		
Days after kindling	29.4	28.0	28.6	28.5		28.7	28.9	28.9		
Doe weight (g)	4431^{a}	4765^{b}	4914^{c}	4968^{c}	<0.001	4841^{c}	4775^{b}	4566^{a}	<0.001	479.7
Doe BCS	1.91^{a}	1.94^{ab}	2.00^{b}	2.09^{bc}	<0.001	1.92^{a}	1.98^{b}	2.01^{b}	0.006	0.496
Litter size (no.)	8.08^{a}	8.21^{a}	8.61^{b}	9.18^{b}	<0.001	8.24^{a}	8.83^{b}	8.19^{a}	<0.001	1.044
Kit weight (g)	541^{a}	575^{b}	540^{a}	554^{ab}	<0.001	616^{b}	641^{c}	369^{a}	<0.001	97.6
Doe health concerns (%)*										
Diarrhoea	7.0	5.2	5.4	6.3	0.096	7.7^{a}	3.5^{b}	6.7^{a}	0.003	-
Pododermatitis	2.8	7.9	1.0	0.0	1.000	4.4	4.5	0.3	0.999	-
Mastitis	2.8	6.1	1.0	0.0	0.080	3.0	1.6	3.7	0.999	-
Dermatomycosis	2.8	3.2	2.8	2.7	1.000	0.0	0.4	9.2	0.999	-
Respiratory sympthoms	0.6	0.2	0.1	0.0	1.000	0.3	0.3	0.3	1.000	-
Litter health concerns (%)*										
Diarrhoea	0.7	0.7	0.7	3.0	1.000	2.4	0.1	0.3	0.999	-
Dermatomycosis	2.4	3.8	0.0	0.0	1.000	0.7	3.9	0.6	1.000	-

RMSE, root mean square error of the model; BCS, body condition score (0: cachexia, 4: obesity). *Percentage of animals affected with respect to the total assessed on each visit per farm. [a, b, c] Means with different letters on the same row significantly differ within housing system or season ($p<0.05$, Bonferroni test).

Table 5. *Risk factors (P-values) for animal-based measures in reproducing does and kits at the pre-weaning visit in farms with different housing systems across three seasons.*

Variation factors	**Housing system**	**Season**	**Animal genotype**	**Reproductive rhythm**	**Parity order**	**Footrest presence**
	Standard/Dual-purpose/ Enriched/Park	Autumn/Winter/Summer	Grimaud/Hyla/Martini	11 d after kindling/ 18 d after kindling	Primiparous/ Pluriparous	Yes/No
Doe						
Live weight	<0.001	<0.001	<0.001	0.37	<0.001	<0.001
Body condition score	<0.001	0.003	0.085	0.013	0.212	<0.001
Diarrhoea	0.066	0.006	0.146	0.207	0.833	0.556
Pododermatitis	0.710	0.001	0.869	0.001	0.029	0.005
Mastitis	0.521	0.042	0.301	0.025	0.55	0.177
Dermatomycosis	0.003	<0.001	0.044	0.013	0.002	0.021
Litter						
Litter size	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Kit weight	<0.001	<0.001	<0.001	0.071	0.233	0.465
Diarrhea	0.679	0.005	0.591	0.509	0.604	0.259
Dermatomycosis	0.999	<0.001	0.122	0.002	0.989	0.991

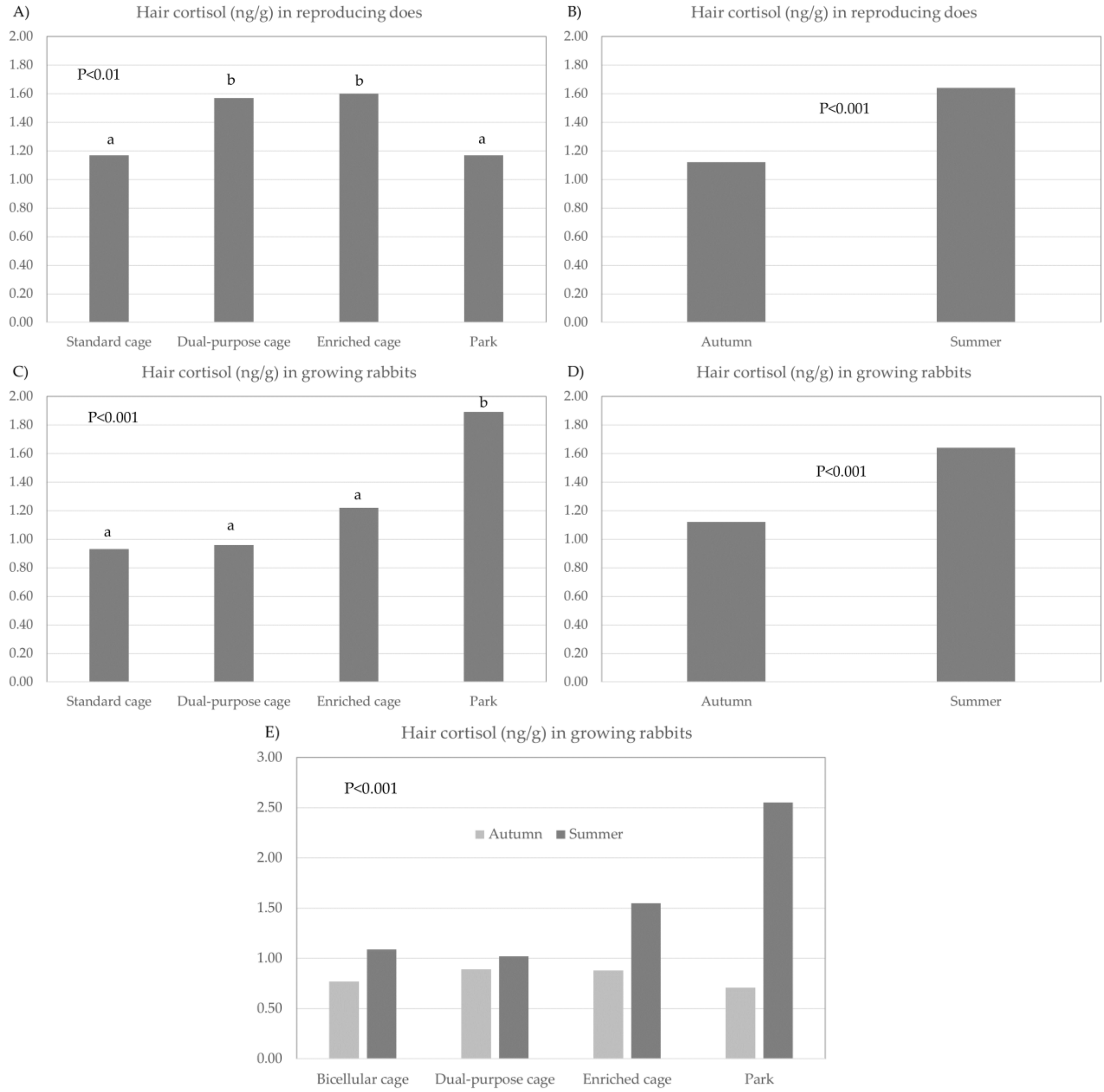

Figure 1. *Hair cortisol content (ng/g): effect of the housing system (A) and the sampling season (B) in reproducing does; effect of the housing system (C), the sampling season (D) and the interaction between housing systems and sampling season (E) in growing rabbits.*

Pre-slaughtering visit

On the visit day, the temperature in the fattening sector was similar among the farms with the different housing systems, while average values for relative humidity and CO_2 were higher in the farms with the standard cage and park systems than in those with the dual-purpose cage housing system. The lowest air NH_3 concentration was recorded in the farms using the enriched cage system (Table 6). Average mortality was numerically higher in the farms with the park system due to the highest value (30.2%) recorded in a single farm on one recording and lower values recorded in the farms using the dual-purpose cage housing system (6.3%) (Table 6).

As for performance, the live weight of growing rabbits decreased from that noted in the farms with the enriched cage to dual-purpose cage system to park and standard cage systems (2584 vs 2509 vs 2464 g and 2456 g; $p<0.001$). Regarding health issues, a higher prevalence of dermatomycosis was found in farms using the park and dual-purpose cage systems in comparison with those using the standard cage and enriched cage systems (32.8 and 32.0 vs 11.2% and 0.3% of controlled rabbits), even though these results were linked to a single farm with a very high dermatomycosis occurrence for both the park and dual-purpose cage systems (Table 6). The prevalence of diarrhea in growing rabbits ranged from 0.0 to 3.5%, while injuries were observed in 0.2 to 8.8% of rabbits, without significant differences among housing systems. As for the season, the growing rabbits had lower body weight in summer than in autumn and they were the heaviest in winter (2332 vs 2558 vs 2619 kg; $p<0.001$). No significant difference was observed concerning health issues among the seasons.

The analysis of risk factors for the performance of growing rabbits confirmed the above-described significant effects in reproducing does about the housing system and season, besides genotype (Table 7; Supplementary Table 3). The season was a risk factor for dermatomycosis as well.

Finally, hair cortisol was higher in the growing rabbits housed in the farms with the parks than in those from other housing systems (1.89 vs 0.93, 0.96, 1.22 ng/g; $p<0.001$; Figure 1C) and was higher in summer than in autumn (1.55 vs 0.94 ng/g; $p<0.001$) (Figure 1D). A significant interaction between housing system × season was observed ($p<0.001$), i.e., the hair cortisol during summer was higher in rabbits from parks than in those from the other housing systems (2.55 vs. 1.09, 1.02 and 1.55 ng/g; $p<0.05$), while no significant differences among housing systems were observed in autumn (0.77, 0.89, 0.88, 1.23 ng/g) (Figure 1E).

Table 6. *Results of the pre-slaughtering visit in farms with different housing systems across three seasons: environmental (means and intervals) and animal-based measures (means) in growing rabbits.*

	Housing system					**Season**				**RMSE**
	Standard	**Dual-purpose**	**Enriched**	**Park**	**P-value**	**Autumn**	**Winter**	**Summer**	**P-value**	
Environmental data										
Recordings (no)	9	8	9	7		12	11	10		
Temperature (°C)	20.1 (13.9-28.1)	19.0 (14.5-24.7)	21.0 (17.6-27.4)	20.3 (16.1-26.5)		18.5 (16.1-21.0)	17.2 (13.9-20.7)	25.4 (21.0-28.1)		
Relative humidity (%)	64.4 (43.2-83.6)	57.4 (43.9-74.0)	62.8 (40.4-77.0)	65.2 (49.5-73.5)		65.8 (55.5-83.6)	54.7 (40.4-74.0)	66.8 (58.6-77.0)		
CO_2 (ppm)	1221 (480-2240)	1063 (520-1567)	1048 (640-1520)	1297 (540-2567)		1334 (600-2233)	1452 (740-2567)	604 (480-680)		
NH_3 (ppm)	8.6 (0.0-21.0)	8.5 (1.2-21.4)	7.2 (3.4-14.0)	8.5 (1.2-21.7)		9.3 (0.0-21.7)	10.9 (1.2-21.4)	3.8 (1.2-6.2)		
Rabbit mortality (%)	8.9 (4.0-12.7)	6.3 (3.8-9.1)	9.0 (1.4-29.9†)	16.5 (7.2-30.2††)		8.5 (2.6-20.8⁑)	15.8 (4.5-30.2††)	7.6 (1.4-15.9)		
Animal based measures										
Rabbits, no.	900	800	900	700		1200	1100	1000		
Age (days)	71.2	70.1	69.2	71.1		70.3	69.8	71.1		
Live weight (g)	2456a	2509b	2584c	2464a	<0.001	2619a	2558b	2332c	<0.001	287.7
Diarrhea (%)*	1.0	0.6	0.0	3.5	1.000	-	13.0	21.7††	0.975	
Dermatomycosis (%)*	11.2a⁑	32.0b†	0.3a	32.8b¥	<0.001	-	0.9	3.1	0.990	
Injuries (%)*	0.2	0.4	0.5	8.8	1.000	-	0.4	1.9	0.999	

†Value recorded in one farm in winter cycle; ††Value recorded in one farm; ⁑Value recorded in one farm with park system; ‡40 animals/cage in one farm

RMSE, root mean square error of the model

a, b, c Means with different letters on the same row differ significantly within housing system or season ($p<0.05$. Bonferroni test)

*Prevalence of health concerns are expressed as the average percentage of rabbits affected with respect to the total number assessed each visit at farm level. Health data of autumn was not analyzed nor showed because they were not comparable with those of winter and summer.

⁑Value associated to one farm with high values in winter and summer; †Value associated to one farm with high values in summer; ¥Value associated to one farm with high values in summer; ††Value associated to two farms, one with dual purpose and the other with park systems.

Table 7. Risk factors (*P-values*) for animal-based measures in growing rabbits at the pre-slaughtering visit in farms with different housing systems across three seasons.

Variation factors	**Housing system**	**Season**	**Animal genotype**	**Reproductive rhythm**	**Feeding system**
Levels	Standard/ Dual-purpose/ Enriched/Park	Autumn/Winter/ Summer	Grimaud/Hyla/ Martini	11 d/18 d after kindling	Ad libitum/ restricted
Live weight	<0.001	<0.001	<0.001	0.132	0.018
Diarrhea	0.427	0.015	1.000	0.996	0.992
Dermatomycosis	0.007	<0.001	0.757	0.975	0.006
Injuries	0.035	0.009	0.868	0.978	0.977

DISCUSSION

The present study aimed to provide new information about the on-farm welfare and health of rabbits. Being under field conditions, the sample size per housing system was low due to the availability of farmers and the low number of commercial farms using alternative systems such as enriched cages and park systems. Therefore, not all production factors were fully balanced among the different housing systems. Due to these limits, we first ran a comparison of farms according to the housing systems, considering the farm with its specific combination of production factors as a random effect; then, we used the risk analyses to elucidate the possible main effects of all production factors. Thus, finally, the tested protocol provided only a preliminary evaluation of rabbit welfare and health in farms using the standard and alternative housing systems, whereas recent on-farm assessments focused on those farms using only standard barren cages (Bignon et al., 2017; Dalmau et al., 2020). Moreover, this pilot study highlighted the troubles of accounting for on-farm rabbit welfare and health exclusively to the housing system.

In fact, being recognized and accepting the complexity of the production systems for rabbits (EFSA, 2020), the health and welfare of reproducing does, and growing rabbits are affected by several factors. Thus, the risk analyses we performed were intended to highlight the role of these factors. The corresponding results are hereby discussed before the comparison of the housing systems.

External factors (such as season), animal-related issues (such as genotype and parity order in does), management, and structure-based factors (reproductive rhythm, presence of footrest in reproducing cages, and feeding system for growing rabbits) played a significant role.

As for the season, performance results in does, kits, and growing rabbits were lower in summer than in autumn and winter. Indeed, rabbits are very sensitive to high ambient temperatures, since they have few functional sweat glands limiting their ability to eliminate excess body heat (Maya-Soriano et al., 2015). Exposure of growing and adult rabbits to severe heat stress adversely affects their growth and reproductive performances as they reduce feed intake to diminish body heat production (Marai et al., 2002; Mousa-Balabel, 2004). The highest hair cortisol levels measured in growing rabbits housed in parks during summer suggest that parks can be more stressful for growing rabbits submitted to heat stress, while in autumn, positive effects due to higher available total surface of parks, higher social interaction, and the presence of a plastic-mesh floor prevail. As for doe health, a higher prevalence of diarrhea was observed in autumn than in the other seasons, which could be due to the susceptibility of rabbits to the sudden temperature and air quality changes that are frequent in this season. Interestingly, the same was not observed with regard to respiratory signs. These changes are the main environmental risk factors for diarrhea as identified also by the experts invited to the EFSA technical hearing meeting (2020). Additionally, both in reproducing does and growing rabbits, dermatomycosis prevalence was much higher in summer than in autumn and winter. Indeed, according to EFSA (2005), dermatomycosis is directly related to environmental factors such as high temperature and humidity, in addition to other factors like low hygienic condition, poor management, and skin lesions (Moretti et al., 1996).

As for animal-related factors, animals belonging to genetic lines selected for growth rate are heavier, have greater feed intake, and better feed conversion than those from lines selected for litter size (Orengo et al., 2009; Sánchez et al., 2012; Ezzerough et al., 2019; Hypharm, 2022). In the present study, Hyla females were heavier than Grimaud and Martini females, the latter being present only in one farm, whereas Grimaud litters were larger and Grimaud kits and growing rabbits were heavier than Hyla and Martini ones (Supplementary Table 2), which is consistent with the observations in the study of Martínez-Bas et al. (2014). Differently, Zita et al. (2012) reported a higher weaning and slaughtering weight in Hyla compared to Grimaud rabbits. Under our conditions, genotype was not associated with any major risk for health issues. In contrast, previous authors (Rosell and de la Fuente, 2013, 2020) found a relationship between genotype and prevalence of pododermatitis in commercial farms with conventional housing systems, with those with the heavier strain at a higher risk of pododermatitis. A relationship with genotype was also previously reported for the prevalence of clinical mastitis in commercial farms (Rosell and de la Fuente, 2018).

As already found in the literature (Trocino et al., 2015), our results showed that performance changed with parity order, with multiparous does being heavier and having larger litters compared to primiparous ones (Supplementary Table 2). Also, in our trial, kit weight and weight gain increased with the parity order of reproducing does due to their higher feed intake and, accordingly, higher milk production. Moreover, based on the literature (Sánchez et al., 2012), the longer the reproductive career, the lower the BCS of the doe. Under our conditions, parity order was also found to be a risk for pododermatitis occurrence but not for mastitis, which is consistent with the results of Rosell et al. (2013).

With regards to management factors, as for the reproductive rhythm, there are several studies comparing doe and litter performance and doe body energy balance using intensive (insemination post-partum or 11-12 days after kindling) or extensive rhythms (insemination after litter weaning), while rhythms based on insemination 17-19 days after kindling have become popular in the field without evidence of data in the literature (Trocino et al., 2015). Under our conditions, in the tested farms using the 11-day rhythm, does had higher BCS and larger litters at weaning than in farms inseminating does 18 days after kindling (Supplementary Table 2), which is quite surprising and would deserve further investigation under experimentally controlled conditions. It could be hypothesized that the ongoing pregnancy in females submitted to the 11-day rhythm accounts for their higher BCS to ensure future offspring compared to females submitted to the 18-days rhythm. Based on the literature (Rosell and de la Fuente, 2018), the reproductive rhythm is a risk factor also for the occurrence of pododermatitis, mastitis, and dermatomycosis in reproducing does. In fact, Rosell and de la Fuente (2018) reported that diseases (mastitis) or worse BCS are predisposing risk factors for infertility, whereas the reproductive rhythm can affect overall farm productive results. Thus, fertility might be included as a further indicator in protocols for on-farm welfare and health assessment. The prevalence of clinical mastitis is also affected by the lactation stage; as in commercial farms, clinical mastitis was found to be more frequent in the fifth week of lactation compared to the first one (Rosell and de la Fuente, 2018).

Our results showed that, among factors linked to housing, the absence of footrest mats was a risk factor for the occurrence of pododermatitis and dermatomycoses in reproducing does, which confirms the usefulness of such a tool (Rosell et al., 2009, 2013; Szendrő et al., 2019).

Welfare and health of reproducing does and litters in different housing systems

We used the criteria established in the Welfare Quality Project (Blokhuis et al., 2010) (Good Feeding, Good Housing, Good Health, and Appropriate Behavior) as a reference for identifying indicators for on-farm measurements. Behavioral concerns and constraints were implicitly assessed by resource- and management-based indicators since there is no doubt about how cage type, group size per cage, and the presence of enrichments can affect movement restriction, resting problems, and expression of social and gnawing behaviors. Moreover, negative behaviors, such as aggression, were assessed based on ABMs, i.e., injuries.

Under our conditions, based on cage dimensions, movement restrictions/resting problems were expected in the standard cage and dual-purpose cage housing systems and to a lesser extent in the enriched cage systems and the single modules of parks for reproducing does. As regards social behaviors, reproducing does were kept with their litters from kindling until weaning, experiencing individual housing for about 7 to 10 days, depending on the reproductive rhythms (kindling to kindling interval: 42 or 49 days; i.e., 17 and 20% of the time covering a reproductive cycle, respectively). Moreover, in the tested farms, does were never kept with other adult mates. Finally, no gnawing object was found in cages or parks of the visited farms for which rabbits were not able to play this species-specific behavior on any farm.

According to EFSA (2020), despite the above-stated differences in available areas for movement, the main welfare consequence for reproducing does in both standard cages and dual-purpose cages, enriched cages, and parks is the restriction of movement, defined as the possibility of performing three consecutive hops. However, again according to EFSA (2020), knowledge is missing about the space requirement to acceptably meet the behavioral and physiological needs of rabbits under farming conditions. Moreover, more space and locomotion possibilities can affect doe performance on-farm (Szendrő et al., 2013, 2019): some authors (Rauterberg et al., 2021) observed higher body weight and weight gain in does housed in conventional cages than those kept in larger cages, while others reported few differences (Szendrő et al., 2013). In the case of reproducing does, an impairment in performance is especially expected when comparing conventional individual housing with collective housing systems, which has been related to aggression and stressful interactions among does rather than to space availability itself (Zomeño et al., 2017, 2018).

Under our conditions, the lowest live weight of the reproducing does in farms with the standard cage system and their lowest body condition score compared to those in dual-purpose cages and, especially, in enriched cages and parks cannot be associated with differences in the genotype distribution or in the reproductive rhythm used or in the distribution of primiparous and multiparous does in the farms using the different housing systems (Supplementary Tables 1, 2). In fact, as for the genotype, as presented above, the heavier Hyla females and the lighter Grimaud and/or Martini females were present in all housing systems. As for the reproductive rhythm, the highest BCS and the largest litters at weaning have been associated with the 11-day rhythm compared to the 18-day rhythm where the former was prevalent in farms with standard cages (two out of three farms) and enriched cages (three out of three farms) compared to farms with dual-purpose cages (one out of three farms) and parks (one out of three farms). Finally, as for the parity order, the percentage of primiparous does used in the evaluation was similar in all farms (10-15% of the total). Nevertheless, the parity order of the doe can play a major role in her status. It would be recommendable to include in the evaluation only does with more than three kindlings, which would represent the majority of the does on the farm and would be in a more stable condition compared to does at the start of their reproducing career. Weaning weight can also affect the adaptability and survival of rabbits after weaning in the growth period until slaughtering (Pascual 2001; Xiccato et al., 2003). In literature, some studies observed worse litter performances in larger cages (Lopez et al., 2019), while others observed heavier kits in larger cages with an elevated platform compared to smaller cages without platforms (Mikó et al., 2014) which was ascribed to a higher disturbance to sleeping of kits due to doe visits in the nest boxes (more than two nursing events/day) in smaller cages. When focusing on health-related welfare consequences in reproducing does, EFSA (2020) ranked heat stress as one of the top five welfare consequences in conventional standard and dual-purpose cages and enriched cages and skin lesions in parks. Indeed, we did not detect any difference in the occurrence of health concerns both for does and litters among the farms with different housing systems. Moreover, a previous study found a higher occurrence of mastitis and diarrhea in larger cages, which was due to the higher soiling of the floor because of an unsuitable footrest mat (Rauterberg et al., 2021).

As for kits, EFSA (2020) ranked hunger as the main welfare concern in conventional cages and parks; neonatal disorders are ranked only for parks, while heat stress, neonatal disorders, and respiratory disorders have been alternatively listed in the three housing systems tested in the present study. However, in the present study, no signs of hunger and neonatal or respiratory disorders were detected, whereas heat stress was likely to occur only during summer in all housing systems, as measured by the low kit weaning weight during this season. In fact, the indoor maximum temperatures we measured during the visits ranged from 24.7 to 28.6°C, which is somewhat higher than the optimal ranges for reproducing does and litter, i.e., 15-20°C, 60-70% humidity (Verga et al., 2007), while severe heat stress occurs above 30°C (Verga et al., 2007; Luzi et al., 2009). Under our conditions, air CO_2 and NH_3 did not exceed the recommended thresholds for farms, i.e., 5000 ppm and 25 ppm, respectively (Wathes and Charles, 1994; Luzi et al., 2009), with the highest values recorded in winter than in summer. This result is consistent with observations of Calvet et al. (2011) and with the Italian climate conditions for which farm air changes are lower during winter to maintain temperature, which results in worse air quality even if always within acceptable ranges (Marai and Rashwan, 2004).

Welfare and health of growing rabbits in different housing systems

As for behavioral constrains in growing rabbits, according to EFSA (2020), inability to express gnawing behavior and resting problems are the main welfare consequences in all the housing systems we compared, while the restriction of movement is ranked in cages but not in parks.

In fact, no gnawing objects were found in cages or parks in which rabbits were prevented from gnawing in all tested farms. Moreover, based on cage size and stocking density (16-17 rabbits/m^2 in standard bicellular cages, dual-purpose cages, and enriched cages; 12 rabbits/m^2 in parks), restriction of movement and resting problems were likely to occur in cage systems compared to parks.

As for the differences found in the final live weight of growing rabbits, considering also differences in slaughter age, the best performance was found in the rabbits kept in farms using the dual-purpose cage and enriched cage housing systems compared to those using standard bicellular cages and parks. These results cannot be attributed to differences in the genotype (since the heavier Grimaud and the lighter Hyla and/or Martini growing rabbits had the same distribution in all housing systems) (Supplementary Tables 1, 3). Also, the nonhomogeneous distribution of the feeding system cannot alone explain the differences in the live weight of growing rabbits in the different housing systems. In fact, the heaviest animals were feed-restricted (two out of three farms with dual-purpose cages) and fed ad libitum (three out of three farms with enriched cages) as it was for the lightest animals which were both restricted (two out of three farms with standard cages) and ad libitum fed (three out of three farms with parks). Even if the best growth performance is not necessarily associated with the best welfare conditions, it is likely that movement restrictions in bicellular cages were too high to favor non-active behaviors and reduce feeding. This hypothesis is supported by the high stocking density (as kg live weight at slaughtering) recorded on farms using standard bicellular cages, i.e., on average 46.0 kg/m^2 (from 33 to 56 kg/m^2), which can support the "prolonged hunger" ranked by EFSA (2020) within the top five welfare consequences for growing rabbits in conventional cages. Moreover, interactions within large groups of animals and high movement possibilities could have reduced feed intake and growth in parks. Indeed, even rabbits kept in small groups have been observed to spend more time moving and less time feeding than rabbits in bicellular cages, which can affect performance (Trocino et al., 2014).

More space and locomotion possibilities, i.e., greater physical activity, can also have a negative impact on performance (Combes et al., 2005; Princz et al., 2009; Gerencsér et al., 2014). However, recent studies showed higher daily weight gain and final live weight in rabbits reared in large groups (58 rabbits) compared with rabbits reared in small groups (12 rabbits) in the first growth period (until 60 days) (Rauterberg et al., 2019). Thus, based also on the low stocking density measured in farms using park housing systems at slaughtering (on average 30.1 kg/m^2; range: 29-32 kg/m^2), a high degree of social interactions due to the group size (32-40 rabbits per group) likely decreased feed intake and growth in parks of the visited farms rather than behavioral restrictions.

As for health concerns, according to EFSA (2020), skin and gastrointestinal disorders are among the top five welfare consequences in rabbits farmed in enriched cages and parks, while in the present study, only a higher prevalence of dermatomycosis was observed in farms using the dual-purpose cage and park housing systems and no effect of the housing system was reported for diarrhea on a small sample size of farms which require confirmation on a larger scale.

At the pre-slaughtering visit, injuries due to aggressive behavior were recorded; the occurrence of injured rabbits was numerically higher in farms using the park system (8.8 vs <1%) but the difference was not confirmed at a statistical level. It is widely reported in the literature that aggressions are positively correlated with increased group size, stocking density, and slaughtering age (Princz et al., 2009; Trocino et al., 2015; Szendrő et al., 2009, 2015). Accordingly, stress is expected to increase with the group size as higher corticosterone levels in hair and feces have been measured in rabbits kept in collective pens compared to rabbits in bicellular cages when age increased (from 63 to 70 days) in previous studies (Trocino et al., 2014). These results are consistent with the increased hair cortisol we measured in growing rabbits housed in parks during summer when temperature/humidity was likely more challenging compared to autumn, as discussed above.

CONCLUSIONS

Despite preliminary testing, because of the low sample size per farm type and the field conditions, the tested on-farm protocol did not highlight major differences in welfare and health of reproducing does and their kits or growing rabbits kept in different housing systems. Few differences for health concerns were recorded among housing systems, whereas neither lesion in growing rabbits due to aggression significantly changed in collective systems with a high group size, such as parks. Importantly, the study outlined the role of several production factors changing from one farm to another, stressing the troubles of accounting on-farm rabbit welfare and health exclusively to the housing system. In perspectives, interactions between these factors and the housing systems should be highlighted to improve the whole production system; on-farm protocols should be refined based on the sensitivity of AMBs to production factors other than the housing system; and ABMs based on feelings should be identified and validated to provide additional tools for evaluating on-farm welfare of rabbits.

SUPPLEMENTARY MATERIAL

Table S1. *Housing systems and cage size in the farms subjected to the on-farm welfare evaluation in reproducing does with their litters and in growing rabbits.*

Farm ID	Cage	Genotype	Reproductive rhythm	Weaning age	Slaughtering age	Footrest mat	Age of cages	Feeding program for growing rabbits	Ventilation Cooling system
A	Standard	Hyla	11	32	84	No	1983	Restriction	Longitudinal No cooling
B	Standard	Grimaud	18	35	80	No	2006	Restriction	Longitudinal Cooling
C	Standard	Hyla	11	37	75	No	1998	Ad libitum	Longitudinal
D	Dual purpose	Hyla	18	35	78-80	No	2010	Restriction	Longitudinal Cooling
E	Dual purpose	Grimaud	18	35	78	Yes	2015	Ad libitum	Transversal Cooling
F	Dual purpose	Hyla	11	37	74-76	No	2009	Restriction	Longitudinal Cooling
G	Enriched	Hyla	11	35	72	No	2017	Ad libitum	Longitudinal Cooling
H	Enriched	Martini	11	35	72	Yes	2008	Ad libitum	Transversal Cooling
I	Enriched	Hyla	11	35	74	Yes	2017	Ad libitum	Transversal Cooling
L	Park	Hyla	11	37	73-75	No	2008	Ad libitum	Longitudinal
M	Park	Hyla	18	35	70	Yes	2015	Ad libitum	Longitudinal Cooling
N	Park	Grimaud	18	38	78-79	Yes	2017	Ad libitum	Longitudinal Cooling

Table S2. *Mean values for animal-based measures in reproducing does and kits at the pre-weaning visit in farms with different housing systems across three seasons based on animal genotype, reproductive rhythm, parity order and footrest presence.*

	Animal genotype			**Reproductive rhythm**		**Parity order**		**Footrest presence**	
	Grimaud	**Hyla**	**Martini**	**11 d after kindling**	**18 d after kindling**	**Primiparous**	**Multiparous**	**Yes**	**No**
Doe									
Live weight (g)	4673	4870	4461	4775	4745	4638	4956	4943	4631
Body condition score	1.93	1.99	1.96	1.98	1.95	1.93	1.98	2.05	1.91
Diarrhea (%)*	3.66	6.87	6.67	6.91	4.58	5.99	5.96	5.44	6.34
Pododermatitis (%)*	1.83	4.71	0.53	1.98	5.14	1.51	7.30	0.33	5.19
Mastitis (%)*	1.50	3.49	2.40	2.15	3.72	1.65	5.41	0.67	4.19
Dermatomycosis (%)*	2.17	1.65	8.26	3.33	2.22	2.92	2.82	1.67	3.70
Litter									
Litter size (no.)	9.16	8.21	8.28	8.35	8.66	8.51	8.57	8.99	8.11
Average kit weight (g)	660	614	549	592	652	612	625	613	618
Diarrhea (%)*	1.83	0.96	0.00	0.67	1.58	1.27	0.48	1.78	0.54
Dermatomycosis (%)*	1.67	2.19	0.27	0.51	3.56	1.01	3.41	2.88	0.00

*Percentage of animals affected with respect to the total assessed on each visit per farm

Table S3. *Mean values for animal-based measures in growing rabbits at the pre-slaughtering visit in farms with different housing systems across three seasons based on animal genotype, reproductive rhythm, and feeding system.*

	Animal genotype			**Reproductive rhythm**		**Feeding system**	
	Grimaud	**Hyla**	**Martini**	**11 d after kindling**	**18 d after kindling**	**Ad libitum**	**Restricted**
Live weight (g)	2567	2448	2375	2459	2468	2512	2418
Diarrhea (%)*	0.60	1.82	0.00	1.43	0.43	1.77	0.00
Dermatomycosis (%)*	0.00	32.6	0.20	14.3	22.9	15.3	20.1
Injuries (%)*	0.20	3.45	0.40	2.79	0.29	3.00	0.25

*Percentage of animals affected with respect to the total assessed on each visit per farm

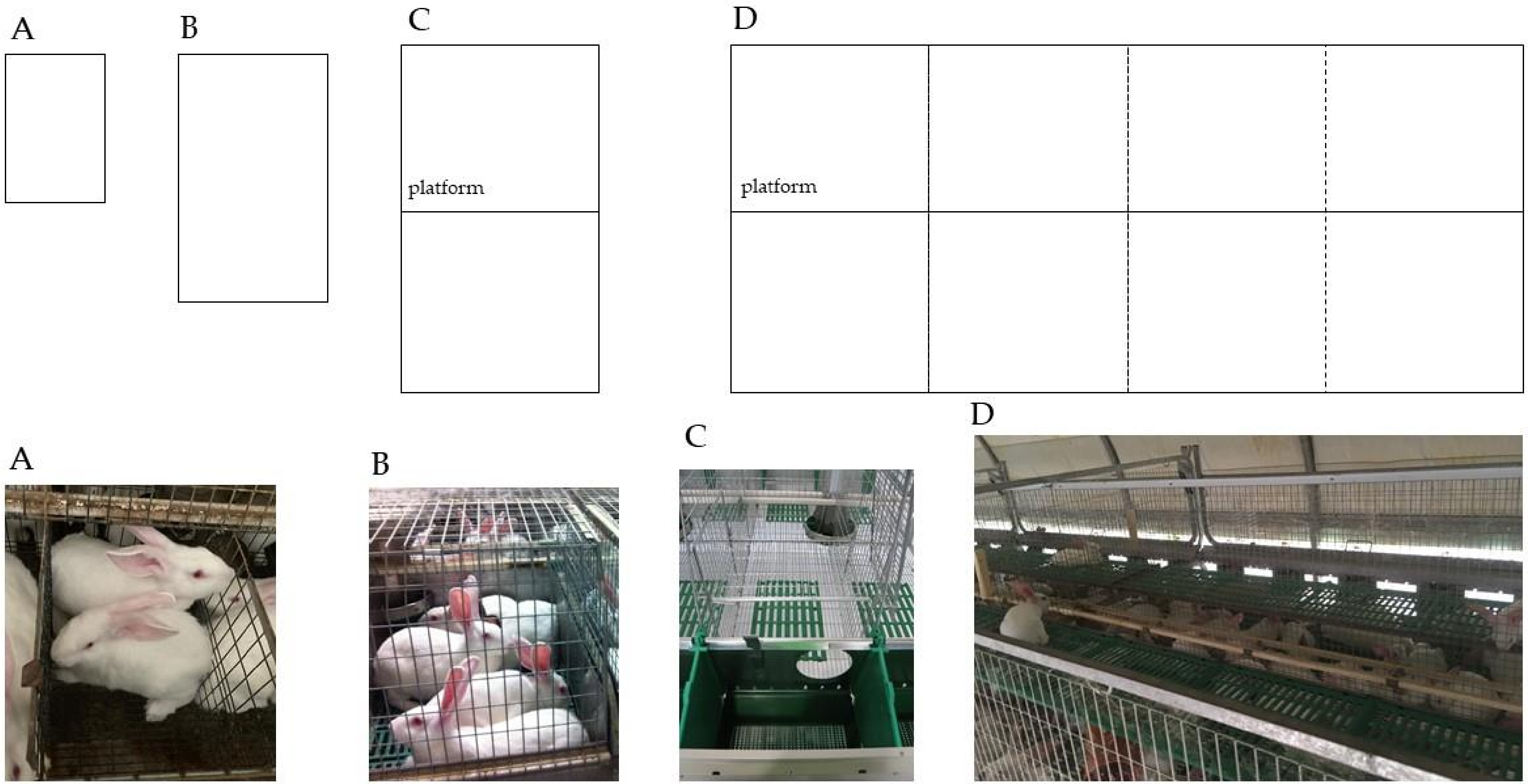

Figure S1. *Schemes and pictures referred to A) Bicellular cage, B) Dual-purpose cage, C) WRSA, D) Park.*

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5. Contribution 3: Movement Restriction in Reproducing Does in a Part-Time Housing System: Effect of Group Size and Grouping Time

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INTRODUCTION

In Europe, rabbits are by far the animals that are mostly raised in cages after laying hens (>112 million per year, respectively). In conventional farms, as for reproducing does with their kits, cage housing systems are based on barren cages and, to a lower extent, on larger and higher enriched cages equipped with a platform. During the last years, park systems (also called elevated pens) have started to be developed, with a few farmers adopting them, where single modules (about 0.5 m2) can be joined for housing reproducing does in continuous or part-time collective systems (EFSA, 2020). In fact, these part-time systems cannot be yet recommended for implementation in the field due to the high level of aggression at each grouping among does and the associated injuries (Szendrő et al., 2019; EFSA, 2020). Different management strategies have been tested to mitigate this negative issue, such as the group size and/or the grouping time after kindling (Buijs et al., 2016; Zomeño et al., 2017, 2018; Martínez-Paredes et al., 2019; Munari et al., 2020; Van Damme et al., 2022).

On the other hand, both in conventional barren cages and in enriched cages, reproducing does, and growing rabbits can suffer movement restriction, defined as the inability to perform three consecutive hops, as the main welfare consequence (EFSA, 2020). Differently, in alternative collective park systems, this welfare consequence is not likely to occur due to the increased functional space available for movement during grouping times.

Indeed, few knowledge is available about the different specie-specific behavioural requirements of rabbits under farming conditions compared to the wild or semi-wild (EFSA, 2020; Rödel et al., 2022) and about the severity (defined as the level of distress and suffering) that is caused by a given related welfare consequence. In fact, the definition of movement restriction has been borrowed from the Council of Europe for the housing of rabbits used for experimental purposes (Council of European Union, 2006) and adopted for reproducing and growing rabbits regardless of differences according to the age and size of animals. Then, as for the behavioural and physiological consequences of this restriction, the only available information about severity is coming from the evaluation by the experts that participated in the EFSA workshop and scored movement restriction on a value of 3 (over a scale of severity from 0 to 9) (EFSA, 2020).

Last, but not least, only one study characterized hopping pattern in rabbits under farming condition as for the number of displacements (Postollec et al., 2006). Then, in reproducing does, other authors (Mugnai et al., 2009; Bignon et al., 2012; Alfonso-Carrillo et al., 2014; Buijs et al., 2015; Zomeño et al., 2018) measured the time spent moving under different housing and management systems.

Thus, the present study aimed at evaluating the hopping pattern in reproducing does kept in a park system to evaluate the occurrence of movement restrictions and the effects of different factors that can affect activity and aggression at grouping among does. In details, the effect of two group/park sizes (2 or 4 does, i.e., 2 or 4 joined modules), grouping time (12 d, 15 d or 18 d after kindling), and the observation day (at grouping time and after 3 days) were evaluated.

MATERIALS AND METHODS

Ethic statement

The study was approved by the Ethical Committee for Animal Experimentation of the University of Padova. All animals were handled according to the principles stated in EC Directive 2010/63/EU.

Experimental facilities, animal management, and experimental groups

The study was performed at the experimental farm of the University of Padova (Italy) between January and April. The farm was equipped with artificial heating and controlled ventilation to maintain environmental temperature (18-26°C) and relative humidity (45-60%) within comfortable ranges. The lighting regime during the whole trial was 16L:8D.

Within the farm, 72 individual modules (53 cm x 92 cm; 0.5 m^2) of a park system were available. Each module was equipped with a plastic floor (rectangular holes: 70 mm length x 12 mm width; distance between holes: 7 mm); two automatic nipple drinkers; a manual feeder; and a removable nest box. The modules could be joined by removing the wire net walls between them, thus forming elevated parks for collective housing of reproducing does.

A total of 112 crossbred nulliparous rabbit does (Hycole, SARL Hycole, Marching, France) were transported by an authorized truck from a commercial farm to the experimental farm. Upon their arrival, they were individually identified by ear marks; does were individually allocated into 72 individual modules of the parks and into 40 dual purpose cages available in the same room. As for the procedures common to all does, one week after their arrival, all does were artificially inseminated. After 14 days, pregnancy was assessed. At kindling, all litters were standardized at 9 kits and does were left free to access the nests during the whole lactation. Nests were removed 23 d after kindling and weaning of litters was performed 31 d after kindling by moving kits in the fattening room of the same experimental farm.

In the individual modules of the parks, the 72 pregnant does were allocated according to a bifactorial arrangement, with two group/park sizes and three grouping times. In details, as for group/park size, two does (N2) or four does (N4) were grouped by joining the same number (2 or 4, respectively) of individual modules at 12 d (T12), 15 d (T15), or 18 d (T18) after kindling. A total of 27 parks was thus obtained with six parks N2 per grouping time (18 parks in total) and three parks N4 per grouping times (9 parks in total). At grouping, the parks N2 were equipped with one plastic platform (53 cm x 35 cm; 25 cm of height from the floor) and one plastic pipe (diameter 16 cm; length 40 cm); the parks N4 had two of the same platforms and pipes (Figure 1).

***Figure 1**. Parks N4, which housed four rabbit does with their kits.*

Video recordings and behavioural data

Each park was equipped with an infrared camera for continuous videorecording (18 Dahua 1080p bullet cameras, focal 2.8 mm, IP 67, IR led, 12 vdc; Dahua Technology Co., Ltd., China). Specifically, 24-h videos were recorded in parks T12, T15, and T18 after joining the modules (at 10:00 a.m.) at 12, 15, and 18 d after kindling, respectively, and then 3 days after grouping per each group (i.e., 15, 18, and 21 d after kindling for T12, T15 and T18 parks, respectively).

The 24-h videos were scored to measure the number of hops on the different areas of the park, i.e. floor, platform, and between platform and floor per doe during the first 30 min of every hour per day of video recordings, for a total observation time of 12 hours per doe per park per day. Hops were identified as single, double, and triple consecutive linear hops. When more than three, the consecutive hops were differentiated as performed in a linear or in a non-linear direction (multi-directions). Movements without complete displacement of the body and hops associated to aggression (to flight or fight) were not considered.

Statistical analysis

Firstly, data were tested for normality with PROC CAPABILITY of SAS (SAS, 2013). Then, the data related to the number and rates of hops were subjected to analysis of variance with PROC GLIMMIX of SAS using the group size (N2 vs. N4), the grouping time (T12, T15, and T18), the observation day (at grouping vs. 3 days after), and the observation hour as the main effects with interactions. The pen was considered as a random effect. Differences between means with $p<0.05$ were considered statistically significant. Probabilities of the effects are given in Table 1.

RESULTS

Across all observations, single hops accounted for 53.72% of all hops observed; double hops were 28.40%, triple hops averaged 12.36%, whereas longer hops accounted for 5.41% (Table 2). Most hops obviously occurred on the floor (83.34%), followed by the hops that rabbits performed to displace between the platform and the floor (15.99%), whereas a few hopping events occurred on the platform (0.56%) (Table 2).

As for the effect of the group size, on average of all the areas, a higher occurrence of single hops was observed in parks N2 compared to parks N4 (56.5% vs 50.74%, $p<0.001$), whereas an opposite pattern was recorded for triple and multiple hops (3 hops: 11.2% vs 13.87%; >3 hops 6.99% vs 3.83%; $p<0.001$). Moreover, as for multiple hops, three and four linear hops were observed in parks N2 and N4, whereas five hops were observed only as "non-linear" consecutive hops in both pens. A low occurrence of six non-linear consecutive hops in N2 pens (0.03%) and N4 pens (0.04%) was recorded. Differences were obviously due to what happened at the level of the floor, where does displace using more hops when kept in groups of 4 compared to groups of 2 (Tables 1 and 2).

As the time of grouping was delayed from kindling, both the number of hopping events (10.18 to 8.12 and 6.92; $p<0.001$) and the occurrence of single hops (59.86% to 50.83% and 53.11%; $p<0.001$) decreased. On the other hand, the rate of double (25.41% vs 30.92% and 28.85%) and triple hops (10.11% vs 13.01% and 13.22%) increased ($p<0.001$) when grouping time was delayed (Tables 1 and 2) which depended on what happened on the floor. Then, the occurrence of (single) hops in the platform decreased when the grouping time increased (1.5% vs 0.4% to 0.27%; $p<0.001$).

As for the observation day, the number of hops per doe during 30 minutes per hour was greater on the day of grouping than on post-grouping (i.e. 3 days after) (9.72 vs 7.09 hops, $p<0.001$). The rates of single (55.79% vs 53.42%; $p<0.05$) and double hops (5.29% vs 4.49%; $p<0.001$) were higher on the grouping day than 3 days after. Moreover, a higher rate of hops on the platform (0.97% vs 0.48%; $p<0.001$) and a lower rate of single and double hops between the floor and the platform were recorded on the grouping day compared to three days later (Tables 1 and 2).

Significant interactions were recorded between the grouping time and the observation day (Tables 1 and 3). In details, the highest number of hops was recorded on the grouping day in the T12 group (12.46 vs. 7.9 on average; $p<0.001$), which corresponded to the highest rate of single hops (62.71% vs. 52.98% on average; $p<0.05$), and the highest number of single hops on the platform. A higher number of hops between the platform and the floor (single, double, and triple hops; $p<0.001$) was recorded in T15 does at 3 days after grouping and in T18 does both at grouping and three days later compared to the other groups (Table 3). As for the interaction between the group size and the observation day (Table 4), some significant differences were recorded for the rates of 4 and 5-non-linear hops, which however were not relevant in absolute terms.

***Table 1**. Probability of the effects of group size (2 vs. 4 does), grouping time (12, 15, and 18 d post-partum), and observation day (at grouping and 3 days after grouping) on locomotory activity (number of jumping events recorded on average in 30 minutes every hour for 24 hours; % of observed events) (means) in rabbit does reared in a part-time collective system.*

		Group size (S)	Group Time (T)	Observation (D)	Observation hour (H)	S*D	T*D
All areas	Totals (n/does per time interval, 30 min)	0.267	<0.001	<0.001	<0.001	0.644	<0.001
	1 hop (%)	<0.001	<0.001	0.014	<0.001	0.434	0.036
	2 hops (%)	0.823	<0.001	<0.001	0.002	0.747	0.005
	3 hops (%)	<0.001	<0.001	0.850	0.003	0.495	0.138
	>3 hops (%)	<0.001	0.357	0.051	0.028	0.673	0.029
F[1]	1 hop (%)	<0.001	<0.001	<0.001	<0.001	0.906	0.002
	2 hops (%)	0.216	0.002	0.325	0.415	0.437	<0.001
	3 hops (%)	0.004	<0.001	0.016	0.008	0.853	0.630
	4 linear hops (%)	<0.001	0.005	<0.001	0.018	<0.001	0.003
	4 non-linear hops (%)	<0.001	0.003	0.236	0.106	0.192	0.991
	5 linear hops (%)	0.001	0.152	<0.001	0.525	<0.001	0.152
	5 non-linear hops (%)	<0.001	0.132	0.120	0.886	0.879	<0.001
	6 linear hops (%)	n.e	n.e	n.e	n.e	n.e	n.e
	6 non-linear hops (%)	0.011	<0.001	0.670	0.296	0.261	<0.001
	>6 linear (%)	n.e	n.e	n.e	n.e	n.e	n.e
	>6 non-linear (%)	0.993	0.003	0.127	0.785	0.208	0.256
P[2]	1 hop (%)	0.707	<0.001	<0.001	0.258	0.174	<0.001
FP[3]	1 hop (%)	0.180	<0.001	<0.001	0.247	0.385	<0.001
	2 hops (%)	0.012	0.014	<0.001	<0.001	0.439	<0.001
	3 hops (%)	<0.001	0.022	<0.001	<0.001	0.046	0.023
	4 hops (%)	0.012	0.091	0.963	0.011	0.194	0.860
	5 hops (%)	0.219	0.950	0.153	0.091	0.778	0.148
	6 hops (%)	0.469	0.635	0.431	0.346	0.798	0.507
	>6 hops (%)	0.968	0.573	0.985	0.364	0.038	0.145

n.e., not estimable

Table 2. *Effect of group size (2 vs. 4 does), grouping time (12, 15, and 18 d post-partum), and observation day (at grouping and 3 days after grouping) on locomotory activity (number of jumping events recorded on average in 30 minutes every hour for 24 hours; % of observed events) (means) in rabbit does reared in a part-time collective system.*

		Group size (S)		Grouping time (T)			Observation day (D)		
		N2	N4	12 d	15 d	18 d	Grouping	Post grouping	RSD
	Totals (n/does per time interval, 30 min)	8.32	8.57	10.18^{c}	8.12^{b}	6.92^{a}	9.72	7.09	1.12
All areas	1 hop (%)	56.5	50.74	59.86^{b}	50.83^{a}	53.11^{a}	55.79	53.42	3.61
	2 hops (%)	28.4	28.39	25.41^{a}	30.92^{b}	28.85^{b}	26.69	30.09	2.94
	3 hops (%)	11.2	13.87	10.11^{a}	13.01^{b}	13.22^{b}	12.23	12	1.94
	>3 hops (%)	3.83	6.99	4.62	5.23	4.81	5.29	4.49	1.31
F[1]	1 hop (%)	48.16	41.51	52.18^{b}	42.19^{a}	43.45^{a}	48.48	43.42	3.68
	2 hops (%)	23.35	24.37	21.57^{a}	25.5^{b}	24.01^{b}	23.39	24	2.78
	3 hops (%)	9.46	10.88	8.38^{a}	10.6^{b}	10.83^{b}	10.55	9.33	1.78
	4 linear hops (%)	0.01	0.27	0.15^{b}	0.14^{b}	0.01^{a}	0.16	0.03	0.13
	4 non-linear hops (%)	2.37	3.84	2.25^{a}	3.27^{b}	3.06^{b}	3.09	2.64	0.94
	5 linear hops (%)	0	0.01	0.01	0.005	0	0	0	0.01
	5 non-linear hops (%)	0.3	1.07	0.62	0.42	0.62	0.63	0.48	0.32
	6 linear hops (%)	0	0	0	0	0	0	0	n.e.
	6 non-linear hops (%)	0.18	0.36	0.44^{b}	0.09^{a}	0.18^{a}	0.27	0.21	0.24
	>6 linear (%)	0	0	0	0	0	0	0	n.e.
	>6 non-linear (%)	0.27	0.26	0.45^{b}	0.25^{a}	0.09^{a}	0.36	0.18	0.31
P[2]	1 hop (%)	0.74	0.38	1.5^{b}	0.4^{a}	0.27^{a}	0.97	0.48	0.59
FP[3]	1 hop (%)	7.73	8.54	6.17^{a}	8.24^{b}	9.4^{c}	6.34	9.52	1.83
	2 hops (%)	5.04	4.02	3.84^{a}	5.42^{b}	4.84^{b}	3.31	6.09	1.56
	3 hops (%)	1.77	2.99	1.72^{a}	2.42^{b}	2.40^{b}	1.68	2.67	0.83
	4 hops (%)	0.55	0.92	0.48	0.85	0.69	0.65	0.7	0.49
	5 hops (%)	0.1	0.18	0.14	0.12	0.12	0.09	0.17	0.19
	6 hops (%)	0.03	0.04	0.04	0.04	0.02	0.02	0.04	0.07
	>6 hops (%)	0.03	0.03	0.03	0.04	0.01	0.02	0.03	0.06

[1] Jumps done on the floor. [2] Jumps done on the platform. [3] Jumps done between platform and floor. [4] * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n.e. = not estimable.

Table 3*. Effect of the interaction between the grouping time (12, 15, and 18 d post-partum) and the observation day (at grouping and 3 days after grouping) on locomotory activity (number of jumping events recorded on average in 30 minutes every hour for 24 hours; % of observed events) in rabbit does reared in a part-time collective system.*

		T12		**T15**		**T18**	
		Grouping	Post grouping	Grouping	Post grouping	Grouping	Post grouping
All areas	Totals (n/does per time interval, 30 min)	12.46	7.9	8.92	7.32	7.77	6.06
	1 hop (%)	62.71^{b}	57.00^{a}	51.81^{a}	49.87^{a}	52.8^{a}	53.43^{a}
	2 hops (%)	21.31^{a}	29.03^{b}	30.21^{b}	31.62^{b}	28.09^{b}	29.61^{b}
	3 hops (%)	9.79	10.42	12.81	13.22	14.1	12.35
	>3 hops (%)	5.69	3.55	5.17	5.29	5.01	4.62
F[1]	1 hop (%)	55.42^{c}	48.93^{b}	46.51^{b}	37.92^{a}	43.47^{b}	43.43^{b}
	2 hops (%)	19.25^{a}	23.91^{b}	27.46^{b}	23.55^{b}	23.49^{b}	24.53^{b}
	3 hops (%)	8.53	8.24	11.29	9.91	11.83	9.83
	4 linear hops (%)	0.22	0.07	0.27	0	0	0.03
	4 non-linear hops (%)	2.48	2.02	3.52	3.03	3.26	2.86
	5 linear hops (%)	0.02	0	0.01	0	0	0
	5 non-linear hops (%)	0.93	0.32	0.32	0.53	0.63	0.60
	6 linear hops (%)	0	0	0	0	0	0
	6 non-linear hops (%)	0.69	0.19	0.08	0.1	0.03	0.34
	>6 linear hops (%)	0	0	0	0	0	0
	>6 non-linear hops (%)	0.64	0.26	0.31	0.2	0.12	0.06
P[2]	1 hop (%)	2.23^{b}	0.77^{a}	0.63^{a}	0.18^{a}	0.03^{a}	0.50^{a}
FP[3]	1 hop (%)	5.06	7.29	4.66	11.78	9.3	9.49
	2 hops (%)	2.57^{a}	5.12^{a}	2.75^{a}	8.07^{b}	4.6^{a}	5.08^{a}
	3 hops (%)	1.26	2.19	1.52	3.31	2.27	2.52
	4 hops (%)	0.48	0.49	0.62	1.08	0.33	0.55
	5 hops (%)	0.16	0.13	0.01	0.23	0.1	0.14
	6 hops (%)	0.05	0.03	0.03	0.05	0	0.04
	>6 hops (%)	0.03	0.04	0.007	0.07	0.03	0

Table 4. *Effect of the interaction between the group size (N2 and N4) and the observation day (at grouping and after 3-5 days) on locomotory activity (number of jumping events recorded on average in 30 minutes every hour for 24 hours; % of observed events) of rabbit does reared in a part-time collective system.*

		N2		**N4**	
		Grouping	Post grouping	Grouping	Post grouping
	Totals (n/does per time interval, 30 min)	9.58	7.06	9.99	7.16
All areas	1 hop (%)	57.44^{b}	55.63^{b}	52.5^{a}	49.02^{a}
	2 hops (%)	26.79^{a}	30^{b}	26.5^{a}	30.27^{b}
	3 hops (%)	11.48^{a}	10.99^{a}	13.73^{b}	14.01^{b}
	>3 hops (%)	4.29^{a}	3.38^{a}	7.3^{b}	6.7^{b}
F[1]	1 hop (%)	50.65^{c}	45.67^{b}	44.1^{b}	38.93^{a}
	2 hops (%)	23.27	23.44	23.63	25.1
	3 hops (%)	10.04	8.89	11.56	10.21
	4 linear hops (%)	0.026^{a}	0	0.444^{b}	0.1^{a}
	4 non-linear hops (%)	2.717^{a}	2.03^{a}	3.83^{b}	3.858^{b}
	5 linear hops (%)	0	0	0.027	0
	5 non-linear hops (%)	0.363^{a}	0.23^{a}	1.16^{b}	0.99^{b}
	6 linear hops (%)	0	0	0	0
	6 non-linear hops (%)	0.233	0.122	0.337	0.385
	>6 linear hops (%)	0	0	0	0
	>6 non-linear hops (%)	0.394	0.137	0.278	0.252
P[2]	1 hop (%)	0.907	0.58	1.09	0.29
FP[3]	1 hop (%)	5.88^{a}	9.38^{b}	7.27^{a}	9.80^{b}
	2 hops (%)	3.52	6.55	2.88	5.17
	3 hops (%)	1.44^{a}	2.11^{a}	2.17^{a}	3.8^{b}
	4 hops (%)	0.46	0.645	1.02	0.824
	5 hops (%)	0.072	0.138	0.127	0.225
	6 hops (%)	0.021	0.032	0.031	0.05
	>6 hops (%)	0.008	0.048	0.047	0.007

The interactions between the observation hours and the other main factors. i.e. group size, grouping time, and observation day, are represented in Figures 2, 3, and 4, respectively. Differences between does in parks with a different group size were erratic and not significantly different (probability of the interaction group size x observation hour > 0.10) (Figure 2). As for grouping time, differences in the number of hops at the different observation hours were greater within does grouped at 12 d compared to those grouped at 15 or 18 d after kindling (Figure 3). Then, differences among does grouped at different times (12, 15, or 18 d after kindling) were greater in the first hours after grouping (which was performed at 10 a.m.) and during the dark period after 1 a.m. (Figure 3). In details, in the first hours after grouping (i.e. at 11 a.m.), T12 and T15 does displayed a higher number of hops compared to T18 does, whereas after 1 a.m. the number of hops remained higher only in T12 does compared to T15 and T18 does (probability of the interaction grouping time x observation hour < 0.05) (Figure 3). As well clear in Figure 4, changes in the number of hops at the different observation hours, and in details the peak around 11.00 and the increase after 1.00 a.m., in Figures 2 and 4 largely depend on the higher number of hops at grouping (12, 15, and 18 days after kindling) rather than what recorded 3 days after grouping at the same observation hours (probability of the interaction observation day x observation hour < 0.001).

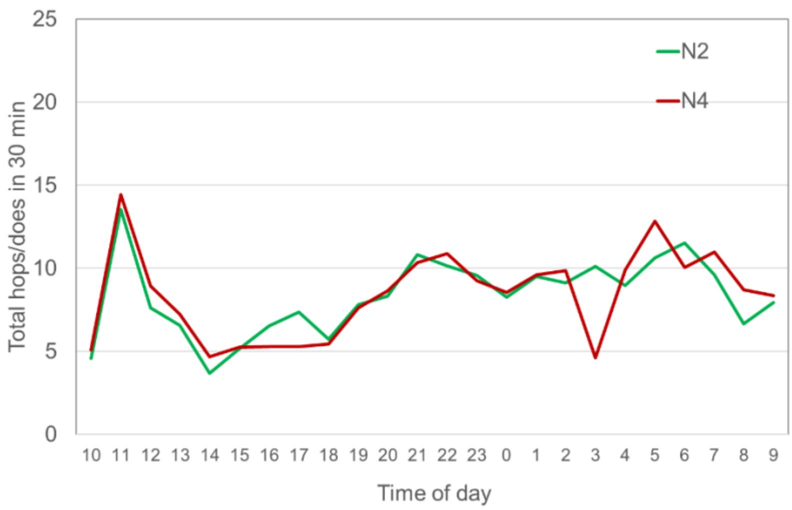

Figure 2. *Number of hops recorded for 30 minutes every hour in rabbit does reared in a part-time collective system with different group size (2 and 4 does per group; N2 and N4) [average of observations at different grouping days (12, 15, and 18 days after kindling) and 3 days after grouping] (probability of the interaction group size x observation hour; p=0.989)*

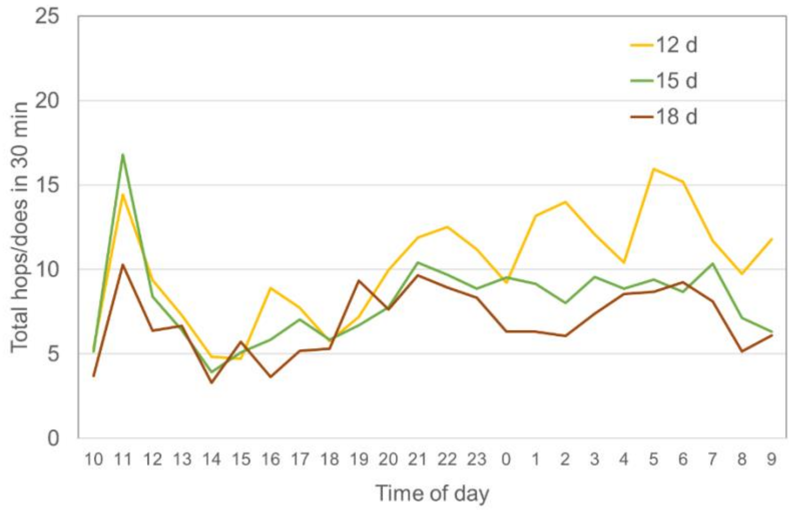

Figure 3. *Number of hops recorded for 30 minutes every hour at different observation hours in rabbit does reared in a part-time collective system with different grouping time (12, 15, and 18 days after kindling) [average of observations of different groups size (2 and 4 does per group) at different grouping days (12, 15, and 18 days after kindling) and 3 days after grouping] (significant probability of the interaction grouping time x observation hour; p=0.032)*

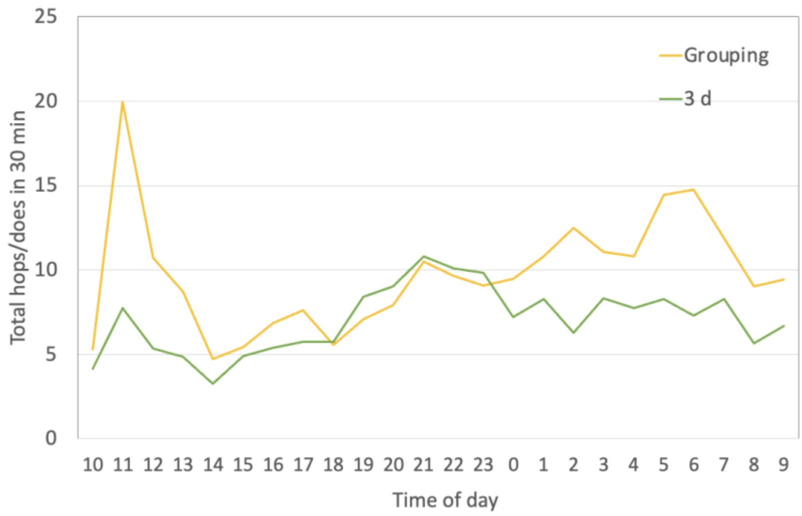

Figure 4. Number of hops recorded for 30 minutes every hour at different observation hours in rabbit does reared in a part-time collective system at grouping (12, 15, and 18 days after kindling) and 3 days after grouping [average of observations of different groups size (2 and 4 does per group)] (significant probability of the interaction observation time x observation hour; p<0.001).

DISCUSSION

Rabbits displace by hops, where a single hop includes pushing of hind legs and landing on the front ones. The length of a single hop can reach 70 cm, depending on body size and the speed of movement, for which an adult rabbit is expected to need at least 70-80 cm in length for one hop and 25-38 cm in width for turning round (EFSA, 2005). Thus, in conventional cages, a movement restriction is obvious, as concluded by EFSA (2020), which can affect the rabbits with a different severity and duration depending on their production stages (kits, growing rabbits, reproducing does) and productive life.

Indeed, while movement restriction has been defined by EFSA (2020) as the inability of rabbits to perform three consecutive hops, few knowledge is available about their behavioral needs and the physiological consequences associated with movement restriction, as it is for several other welfare restrictions in rabbits. Furthermore, few studies focused on the jumping pattern of growing rabbits under farming conditions (Postollec et al., 2006), whereas no specific information exists to our knowledge in reproducing does.

Indeed, based on the budget time, regardless from the housing system (single or part-time) and the observation time (at grouping or later for part-time systems), reproducing does have been found to spend most of their time resting and/or in stationary behaviours (from 68.5% to 82.3% total time of observation) by Buijs et al. (2015) which was confirmed as a range also by other Authors (Alfonso-Carrillo et al., 2014; Rommers et al., 2014). Indeed, under natural conditions, rabbits rest for 12-18 h a day despite not continuously (EFSA, 2005). Then, the other behaviours that mostly impact on the budget time of the rabbits under farming conditions are grooming and feeding to a different extent depending on the type of housing, the management conditions, and the observation time (feeding from 5.56% to 16.2% observed time; Buijs et al., 2015).

On the other hand, time spent in hopping or running has always found to be rather low both in smaller individual systems (0.11% to 0.25% of total observation time; Buijs et al., 2015) and in larger semi-group systems (0.17% to 1.41% of total observation time at 12 d after group formation, Buijs et al., 2015; 2% of total observation time at 4 d after group formation, Rommers et al., 2014). These results have been confirmed by Van Damme et al. (2022) who reported an average time spent in locomotion (not triggered by other does) ranging 1.28% to 1.61% of total observed time in rabbit does grouped at different days after kindling in a part-time system. Nevertheless, the increase of the cage size is associated to an increase of the time spent in active behaviours and a decrease of the time spent on stationary behaviours. In fact, Bignon et al. (2012) found that young does (from 11 weeks of age until weaning of their first litter) increased the time spent in active behaviours (standing, sitting, and moving: 8.3% vs 12.2% and 15.9%; $p<0.001$) and decreased lying time (63.8% vs 57.5% and 52.0%; $p<0.05$) when housed in wider and enriched cages (intermediate size, 33 × 68.5 × 40 cm or large size: 38 × 90 × 60 cm with a platform of 35 × 25 cm at 30 cm height) compared to standard cages (25 × 46 × 28.5 cm).

Observations from the present trial about the number of displacements by hops indeed confirm that the locomotory activity of rabbits under farming conditions is rather low despite the increase of available space from parks N2 (100 cm length x 90 cm depth) to parks N4 (200 cm length x 90 cm depth). Also, in both parks, rabbit does were able to perform 3 linear hops, whereas 4 and 5 linear hops were physically feasible but they were only occasionally observed (0.20 and 0.33 events per doe in 30 min as for 4 linear hops in parks N2 and N4, respectively; 0.03 and 0.09 events per doe in 30 min as for 5 linear hops in parks N2 and N4; data calculated from data available in tables). In other words, a length of 100 cm does not prevent 3 linear hops and, thus, does not produce a movement restriction as defined by EFSA (2020). On the other hand, it is worth to note that observations of the present trial refer to standard displacement of rabbit does, i.e. locomotion not associated to aggression and thus at rather quiet speed. Nevertheless, the increase of the group size (i.e. the park size) decreased the rate of single hops and increased that of multiple hops which is logical and consistent with previous results in growing rabbits where more extreme housing conditions have been compared. In details, the frequency of runs, hops, and consecutive hops have been found to be significantly higher in growing rabbits kept in large pens (3.67 m^2, 50 rabbits) compared with rabbits kept in small pens (0.66 m^2, 10 rabbits) or in conventional standard cages (0.39 m^2, 6 rabbits) (Postollec et al., 2006).

Under our conditions, on average of the two park sizes, single hops were more than half of the total recorded hops. Rather than limitations in the total length for consecutive hops, the presence of kits could have reduced multiple hops when they were out of nests (i.e. observations after 18 day of age). On the other hand, the presence of PVC tubes was not an issue to this regard as they were positioned along the main length of the pen (Figure 1). Then, the presence of platforms permitted further hops between the different levels (i.e. the floor and the platform) as already reported (Alfonso-Carrillo et al., 2014) which also permitted does to escape and/or isolate from conspecific after grouping and from kits later when they were out of the nests. Indeed, the small platforms (35 x 54 cm length) used in this trial did not allow free movement and more hops than single ones over them.

As for the effects of the factor tested to mitigate aggression at grouping, i.e. grouping time, rabbits grouped after 12 days post-kindling displayed a higher number of hops, especially single hops, compared to those grouped later (15 and 18 days after kindling). These results are consistent with previous observations where does grouped earlier spent a higher time in locomotion compared to later groupings (grouping at 22, 25, and 28 d after kindling in Van Damme et al., 2022).

Then, in our trial, on average of different grouping times, the locomotor activity measured as number of hops was greater on the grouping day compared to 3 days later. In fact, Zomeño et al. (2017) reported a reduction of negative interactions (and thus activity of rabbits) within few hours after grouping already in the first 24 h. Group formation is thus associated with higher locomotion as Buijs et al. (2015) reported an increase (locomotion until 5% of observation time) in a part-time system on the first day of group formation because of the behaviours related to the hierarchy formation among does. Nevertheless, despite a reduction of aggressive behaviours is expected, Van Damme et al. (2023) reported a reduction of the injuries score in reproducing does but an increase in kits as time passed after grouping.

In the present trial, differences in the number of hops according to the observation hour (despite the significant interactions with group size and grouping time) depended both on the hours after grouping and the circadian rhythm of activities in rabbits. In fact, the main activities (feeding and locomotion) of wild rabbits are concentrated around morning and dusk (EFSA, 2005), which is consistent with the higher number of hops observed after 1.00 a.m. in the present trial after the first peak observed at 11.00 a.m. and due to grouping (connection of individual modules and formation of parks at 10.00 a.m.).

Overall, a collective housing system (both continuous and part-time) has been found to reduce movement restriction compared to conventional individual caging (Buijs et al., 2015; Dal Bosco et al., 2019), but cannot be recommended because of the high occurrence of aggression among does, lesions and mortality of kits (EFSA, 2020; Van Damme et al., 2023; Trocino and Xiccato, 2024).

During the last years, the evaluation, and the assessment of animal welfare in livestock as in rabbits have been largely based on negative conditions and indicators, such as fear and pain, behavioural restrictions (including movement restriction) and abnormal behaviours, diseases and/or pathological conditions (Boissy et al., 2018). Accordingly, legislation and protocols for assessing welfare on farm have been developed to protect animals against these negative issues (Voogt et al., 2023). On the other hand, recent research is exploring the possibility for the animals to perform positive welfare-related behaviours and/or to have a positive emotional state (Paulovic et al., 2024). However, nor the definition nor the assessment of positive welfare are yet definitively stated and agreed among scientists, where a multidisciplinary approach is called to this purpose (Paulovic et al., 2024). Surely, understanding the biological basis of animals' behaviour in farming conditions is compulsory for feasible and valid protocols to evaluate animal welfare, especially for rabbits as for this species few information is yet available (EFSA, 2020), where the present study has been designed as a first contribution to this regard.

To our knowledge, a first review about the positive welfare indicators that could be used in rabbits, regardless from why and how they are kept (lab, meat, other uses), has been published by Cohen and Ho (2023) which largely focused on specie-specific and functional behaviours of rabbits in the wild conditions without any reference to locomotion activity. As for reproducing does, which life span is minimum one year, a movement indicator such as the number of hops performed by rabbit does within the housing space, when validated, might be able to discern whether the rearing environment respects the physical and behavioural movement needs of the does. Indeed, some indicators of negative emotional response (i.e. the length of time their ears are pressed back and eyes are closed, a higher frequency of self-grooming, and higher frequency of the freeze reaction) have been identified in rabbits used for pet therapy during animal assisted interventions compared to situations in which the animal has the opportunity to retreat (Součková et al., 2023), which could be used to get information about the emotional response of rabbits under other conditions after proper validation.

CONCLUSIONS

The results of the present study confirm that, locomotion activity of reproducing rabbits under farming conditions is rather low regardless from the space availability. A length of 100 cm does not produce a movement restriction, i.e. impossibility of performing 3-linear hops when movement is associated to normal displacement and not related to aggressive events.

Nevertheless, the increase of the park size increases the occurrence of multiple hops which, however, under our conditions could also be related to the higher interactions in the larger pens with four does compared to the smaller pens with two does. Future research should be designed to test the emotional response of rabbits with different possibilities of movements to validate if the number of hops/the locomotory activity can be used as a positive welfare indicator as it would not merely measure the absence of suffering, but it would emphasize the presence of positive experiences.

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6. MAIN CONCLUSION AND PERSPECTIVES

This thesis not only sought to improve our understanding of the welfare and health of rabbits raised in cage-free systems.

Cage-free housing systems, commonly referred to as parks, could offer a fundamental advance in improving rabbit welfare by facilitating greater physical activity and interaction with a wide range of environmental enrichments. However, it is critical to recognize that group keeping in such systems can precipitate aggression issues among rabbits and health problems, particularly related to suboptimal hygiene practices associated with certain enrichments such as platforms.

Although the introduction of blocks of hay for gnawing has demonstrated its positive impact on some aspects of rabbit behavior and welfare, the effectiveness of this strategy remains dependent on complex variables, such as gender group composition.

Furthermore, the on-site health and welfare assessment highlighted the multiple challenges inherent in raising rabbits within cage-free systems. This highlights the critical importance of careful monitoring and targeted management practices to address these specific challenges.

In particular, our study reported original insights into the hopping behavior of reproducing does when kept at different group size and under different group management, where based on observations of the occurrence of single and multiple hops in smaller and larger pens, single hops are mainly displaced followed by double and triple hops. Under the tested conditions, the occurrence of multiple hops (more than 3 consecutive hops) was very low where it remains to be assessed whether this result is associated to the proximity of resources (feed, water, enrichments), the particular set up of the pens (with corresponding enrichments) and/or to the intrinsic nature of the rabbits.

The process of renovating agriculture and animal production in Europe, which began with the Green Deal of the European Commission, is unlikely to be consequential for the livestock sector. The most impactful thing for the survival of the sector will be the change in the housing system that the sector will have to face, whether through national or European laws, or through the law of the market and the positions of consumers. On the other hand, the change of the accommodation system will imply other improvements in the production system which are key for social acceptance and the viability of this production, with respect to what consumers could request the improvement of other ethical aspects.

Based on the information gained from investigations so far, the transition could be gradually made more simply for growing rabbits, as risks in alternative systems such as aggression, health and production are known, as are mitigation strategies. On the other hand, a change in the system of allocation of the rabbits in reproduction of collective systems is not at this moment sustainable due to the negative consequences on the welfare of the rabbit does and their kits. New lines of investigation to improve welfare of the concepts in large areas should consider the opportunity of identifying strategies and experiences that can be positively influenced by the concepts and improvements, in a complete balance, on the quality of life, as you are investigating in others animal species and production.

In essence, this thesis contributes to the existing body of knowledge on the welfare and health of rabbits in cage-free systems. However, it also highlights the existence of numerous unanswered questions in this area. Therefore, there is an urgent need for further research aimed at refining housing and management strategies, ensuring their effectiveness in promoting animal welfare and determining their practicality on a larger scale.