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Welfare and productivity of growing rabbits in collective housing systems

Ph.D. Thesis

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Contents

Abstract

Public opinion asks for adopting welfare-friendly techniques during rearing of farm animals. The housing system in pairs within wire-net cages (2 rabbits/cage) or small groups (4-8 rabbits/cage), which is currently implemented for rabbits should change and alternative pen housing should be adopted to ameliorate animal welfare in respect of growth performance and meat traits. The farming environment should change and be adjusted to the presence of a large group of animals with diversified social relationships that could be influenced by gender composition and slaughter age. The passage from the conventional housing systems to the new alternative ones cannot be achievable without previous research on the effects of this new system on growth performance and welfare of animals.

To achieve this general goal, four trials have been conducted under the present thesis and the summaries of the results obtained are reported below:

Trial No. 1-*Welfare and productivity of growing rabbits housed in bicellular cages and collective pens*

The aim of this study was to measure growth performance, behaviour, fear and stress levels of rabbits reared in bicellular wire-net cages (2 rabbits/cage) compared to those kept in open-top collective pens with wooden slatted floor (sections of the slat: 8 × 8 cm; distance between slats: 3 cm). The effect of pen size (small vs. large) and stocking density (12 vs. 16 rabbits/m^2) in the collective pens was also evaluated. To this purpose, a total of 456 Hyplus crossbred rabbits were reared from weaning (35 d of age) to slaughter (77 d) in 40 bicellular wire-net cages (28 × 40 × 28 cm; 2 rabbits/cage; 18 animals/m^2); 4 small collective pens (1.40 × 1.20× 1.05 m-height) at low stocking density (20 rabbits/pen; 12 animals/m^2); 4 small collective pens at high stocking density (27 rabbits/pen; 16 animals/m^2); 2 large collective pens (1.40 × 2.40 × 1.05 m-height) at low stocking density (40 rabbits/pen; 12 animals/m^2) and 2 large collective pens at high stocking density (54 rabbits/pen; 16 animals/m^2).

The housing of rabbits in collective pens decreased final live weight (2839 vs. 2655 g; P<0.01), daily weight gain by 10.0% (P<0.01), and feed intake by 10.7% (P=0.001), compared with rabbits housed in bicellular cages, without affecting feed efficiency. The rabbits kept in collective pens rested more than those in bicellular

cages did (82.1 vs. 77.6% of observed time; P<0.01), spent less time feeding (7.8 vs. 10.9%; P<0.01), allo-grooming (0.65 vs. 1.58%; P<0.001) and more time moving (0.81 vs. 0.35%; P<0.001). The percentage of rabbits sensitive in the tonic immobility test was lower in rabbits kept collectively compared to those in bicellular cages (76.6 vs. 93.8%; P=0.03). In the open field test, the rabbits kept in collective pens moved less (40.4 vs. 57.2 s; P<0.001) and explored the arena for a shorter time (345 vs. 371 s; P<0.001) than those housed in bicellular cages; instead, the latter stood still for a longer period of time (82.1 vs. 38.7 s; P<0.001). The rabbits kept in collective pens had higher hair corticosterone level (15.7 vs. 6.7 ng/g for rabbits from the cages; P<0.001). Within collective housing systems, stocking density had no effect on behaviour; whereas increasing pen size numerically reduced the time rabbits spent eating (8.30 vs. 6.55%), self-grooming (6.39 vs. 5.47%) and resting (81.1 vs. 84.1%).

In conclusion, rabbits housed in collective pens displayed a more complete behavioural pattern, despite resting more; they were bolder toward humans, but more fearful in a new environment than rabbits from cages. In the tested conditions, pen size and stocking density within collective systems exerted only a weak effect on behaviour, fear and stress levels of meat rabbits.

Trial No. 2 - *Effect of floor type, stocking density, slaughter age, and gender on welfare and productivity of growing rabbits housed in collective pens*

At 34 d of age, 376 crossbred rabbits of both sexes were collectively (20-27 animals) housed in 16 open-top pens (0.12 × 0.14 × 1.05 m-height; 1.68 m^2) according to a 2×2×2 factorial arrangement with two types of pen floor (wooden vs. plastic slatted), two stocking densities (12 vs. 16 animals/m^2), and two slaughter ages (76 vs. 83 d). The wooden slatted floor had slat dimensions: 8 × 8 cm and distance between the slats: 3 cm; whereas, the plastic slatted floor had grid dimensions: 7 cm length and 1 cm width and distance between the grids: 0.7 cm. The rabbits were examined for growth performance, behaviour, reactivity and stress level.

The type of floor influenced productivity: rearing rabbits on a plastic rather than a wooden slatted floor promoted slaughter weight (2795 vs. 2567 g; P<0.001). Concerning behaviour, the rabbits kept on wooden floor spent less time allo-grooming (0.18 vs. 0.25%), running (0.01 vs. 0.07%), biting (0.06 vs. 0.14%) and scratching (0.03 vs. 0.06%) the pen and performed less hops (0.01 vs. 0.07) than rabbits kept on plastic floor (0.001<P<0.09); besides, time spent resting with the legs gathered under the body was higher, while with legs stretched was lower in rabbits on wooden floor compared to the rabbits kept on plastic (41.3 vs. 35.1% and 27.1 vs. 31.9%; P<0.001, respectively). At the tonic immobility test reaction was similar in both groups, whereas behaviour was different at the open field test: the rabbits kept on wooden floor tended to be less prone to enter spontaneously into the arena (60.0 vs. 72.5%; P=0.09) and they were less active: they moved less (26.0 vs. 31.2 s; P=0.03) and passed fewer times by the central square (0.48 vs. 2.27; P<0.001) than rabbits kept on plastic floor, they spent less time grooming (1.94 vs. 4.25 s; P<0.01) and standing still (35.1 vs. 52.7 s; P=0.02) and they adopted an alert position less frequently (0.96 vs. 2.32; P<0.01). Additionally, they performed more digs (0.44 vs. 0.13; P<0.01) and hops (1.57 vs. 0.84; P=0.05). During the novel object and human contact tests, the rabbits reared on the wooden floor were less interested in contacting the object or the man than those on the plastic floor (50.3 vs. 87.2% and 24.0 vs. 48.1%, for the two tests respectively; P<0.01). Values for faecal corticosterone were higher in rabbits kept on the wooden floor compared to those on plastic (41.7 vs. 37.8 ng/g), but no significant (P=0.06).

Increasing stocking density from 12 to 16 rabbits/m^2 significantly impaired daily growth (40.2 vs. 38.7 g/d; P<0.01), feed intake (119 vs. 123 g/d; P<0.01) and final live weight (2725 vs. 2637 g; P<0.01). Rabbits at the higher stocking density increased time spent resting (66.6 to 68.9%) and with body crouched (35.5 to 40.8%) and decreased time spent self-grooming (16.1 to 14.9%) and sniffing (4.49 to 4.11%) their pen (0.001<P<0.06). With the increase of the stocking density, the duration of rabbits immobility at the tonic immobility test, was reduced (68.2 to 49.0 s; P<0.01); whereas, during the open field test, rabbits were less prone to enter spontaneously into the arena (58.8 vs. 73.8%; P=0.4) and tended to move less (30.8vs. 26.3 sec; P=0.07). Rabbits reactivity during the novel object test was not affected; whereas during the human contact test rabbits at the higher density only tended to contact man more times (41.3 vs. 28.0%; P=0.08).

When slaughter age increased (from 76 to 83 days), live weight at slaughter and feed intake increased (2574 vs. 2788 g and 119 vs. 123 g/d, respectively; 0.001<P<0.02); whereas, daily weight gain was impaired (40.0 vs. 38.6; P=0.01). The age at the time of video-recording highly influenced behavioural budget time of animals. As rabbits were getting older, they diminished time dedicated in feeding and drinking (11.0 to 6.94% and 1.81 to 1.46%; P<0.001, respectively), in self- and allo-grooming (16.7 to 14.3% and 0.27 to 0.16%; 0.001<P<0.01, respectively), in running and moving (0.06 to 0.01% and 1.61 to 1.20%; 0.001<P<0.01, respectively) and in sniffing (5.49% to 3.36%; P<0.001). Additionally, they performed fewer times hopping and rearing (0.19 to 0.01 and 0.14 to 0.003; P<0.001, respectively) compared to the younger ones. Contrary, older animals augmented time spent in resting (63.0 to 72.8%; P<0.001). With the increase of age, rabbits were less reactive during the open field (less total displacements: 38.0 to 33.9, exploration: 380 to 365 s, running: 12.0 to 3.27 s and grooming: 4.42 to 1.87 s; P<0.001) and tonic immobility tests (duration of immobility reduced 63.7 to 52.4; P<0.001). Similarly, the older rabbits were less keen on contacting the object (78.1 to 59.4%) and man (43.5 to 26.5%) during the novel object and the human contact tests (P<0.001).

The percentage of rabbits with lesions (due to aggressions) varied with stocking density (8.2 vs. 26.2% for 12 vs. 16 animals/m^2; P<0.001), slaughter age (15.0 vs. 22.2% at 76 vs. 83 d; P=0.07), and gender (11.3 vs. 25.8% for females vs. males; P<0.001).

In conclusion, the growth performance of pen-housed rabbits was largely determined by the type of floor and less affected by stocking density. Rearing on plastic slatted floor resulted more comfortable for the rabbits, leading to lower fear and stress levels. The differences in the percentages of wounded animals due to experimental factors deserve further investigation from the perspective of animal welfare issues.

Trial No. 3–*Effect of environmental enrichment and slaughter age on welfare and productivity of growing rabbits housed in collective pens*

The aim of this study was to evaluate the effect of environmental enrichment and slaughter age on welfare and productive performance of 504 rabbits reared from 33 days of age until slaughter in 16 collective pens (0.12 × 0.14 × 1.05 m-height; 1.68 m^2) with plastic slatted floor (grid dimensions: 7 cm length and 1 cm width; distance between the grids: 0.7 cm). Two rearing systems were used: half of the pens were equipped or not with a plastic slatted platform (50 × 120 cm; 30 cm above the plastic floor) and, within each group, half of the pens were additionally equipped or not with a plastic tube (ϕ 20 cm; length: 50 cm) for hiding. Finally, half of the rabbits were slaughtered at 68 days and the remaining half at 75 days of age.

Growth performance at the end of the trial was not influenced by the presence of the platform, whereas the percentage of rabbits with injuries on genitals before slaughter was significantly higher in rabbits reared in pens with platform rather than in those without (20.6 vs. 11.7%; P<0.01). The presence of the tube negatively affected growth performance in the whole period of rearing (daily growth rate: 39.7 vs. 43.4 g/d for presence vs. absence of tube; P<0.001) and the final live weight (2467 vs. 2600 g for presence vs. absence of tube; P<0.01).

Tube did not alter rabbits behaviour; whereas platform influenced their budget time (0.001<P<0.05): rabbits spent more time resting with their body stretched (33.7 vs. 31.4%), feeding (11.3 vs. 8.49%), and manifesting more species-specific behaviours (licking and biting: 0.37 vs. 0.21%, alerts 0.13 vs. 0.06%) in the pens with platform. The enrichment elements did not affect reactivity of the rabbits during the open field and tonic immobility tests. Tube affected hair corticosterone level which was higher in rabbits kept in pens with tube (8.06 vs. 6.74ng/g; P=0.02).

When the age of rabbits at slaughter was increased, the final live weight of animals increased (2467 vs. 2600 g; P<0.01), while the daily weight gain decreased (43.8 vs. 39.2 g/d; P<0.001). The percentage of animals with injuries on genitals tended also to increase (15.6 vs. 18.0%; P=0.08). Older rabbits dedicated more time in resting with their body crouched (33.2 vs. 27.8%), in self-grooming (17.7 vs. 13.4%) and sniffing (5.24 vs. 3.71%) and less in manifesting species-specific behaviours (0.98 vs. 1.23%) (P<0.001). During the open field test (0.001<P<0.02), older rabbits passed fewer times by the centre of the arena (3.41 vs. 4.25) and they explored it for more time (534 vs 511 s); also, they were running (7.66 vs. 5.48 s), grooming (6.99 vs. 6.75 s) and biting elements of the arena (11.7 vs. 2.87 s) for more time. Older rabbits presented higher hair concentrations of corticosterone (P<0.001): 8.73 vs. 6.07 ng/g.

Gender affected productive performance: males grew less than females and were lighter at the end of the experimental period (2508 vs. 2559 g; P=0.02). The percentage of rabbits that showed lesions at genitals was higher in males than in females (29.3 vs. 4.74%; P<0.001). During the open field test females were less active (latency to enter: 24.0 vs. 19.7 s; standing still: 57.7 vs. 45.6 s; grooming: 6.03 vs. 7.84 s; biting: 5.16 vs. 6.51 s) within the arena than males (P<0.001). Corticosterone level in hairs was not changed between the sexes.

In conclusion, rearing in pens equipped with platform allows the increase of the number of housed rabbits irrespective of the available space per animal without negative effects, whereas the tube impairs productivity and stress of animals. With the increase of slaughter age negative effects become more intense, which is accompanied by an increase of aggressiveness among rabbits. At last, after clarifying some points still uncertain, rearing separately by gender in large groups as a way to diminish the incidence of injuries would be a new possibility of alternative pen housing for growing rabbits.

Trial No.4–*Effect of different gender composition and floor combinations on welfare and productivity of growing rabbits housed in collective pens*

A total of 288 crossbred rabbits were reared from weaning to slaughter (33-74 days of age) in 18 collective pens each including 16 animals and controlled for growth, behaviour, reactivity and stress. The pens were consisted of two modules (each of them: 64 cm-wide, 78 cm-long, 110 cm-high, usable area: 0.5 m^2/module) connected to each other by a hole (23 × 25 cm) and equipped with different types of floor creating three combinations: steel slat/plastic slat vs. steel slat/wire-net vs. wire-net/plastic slat. The galvanised wire-net had hole dimensions: 10 mm-wide, 70 mm-long and diameter: 3 mm; the steel slatted floor had quadratic slats put transversely to the pens with 20 mm-section and distance between the slats equal to 15 mm; and the plastic slatted floor had slats: 70 mm-long, 10 mm-wide and distance between slats equal to 7 mm. Rabbits were kept in groups of separated gender or in mixed-gender groups (only females vs. only males vs. mixed).

Growth performance was not affected by the experimental factors. Rabbits showed a clear preference towards the different floors: on average, 5.57 rabbits were observed to stay in modules with steel slat floor, 7.79 rabbits in modules with wire-net and 10.6 rabbits in modules with plastic slat ($P<0.001$). However, from 59 days of age the distribution of animals was more equilibrated in the two modules of each pen, as with the increase of body weight occupance of space augmented.

The analysis of ethograms (at 42, 56 and 69 days of age) in pens with different floors confirmed animals preference to rest and to perform their activities in the module with plastic slat floor (when it was present) rather than wire-net and steel slat floors. During the open field test, rabbits reared in modules with wire net/plastic floor showed a more active behaviour than those kept on the other floors: they entered more spontaneously into the arena (64.7%, 30.6%, 33.3% of rabbits kept in pens with wire net/plastic, steel/wire net, and steel/plastic floors, respectively; P=0.01), they crossed a higher number of squares (42.4, 30.4, 30.1; P=0,03), run for a longer time (3.59 s, 1.72 s, 1.78 s; P=0.03) and jumped more times (0.47, 0.19, 0.25; P=0.05).

At 42 days of age, females demonstrated a clear preference for staying more on one module (77.5%), tending to remain grouped together compared to males (74.8%) and mixed groups (71.7%) (P=0.03). Less strong was the effect of the gender composition on budget time during the first ethogram. The number of hops resulted significantly higher (P=0.05) in the female pens (1.31) compared to male (1.01) or mixed-gender pens (1.04). However, in the male pens time spent moving was lower (1.58% in male pens, 1.90% in femals pens, 1.84% in mixed pens; P=0.04). At 56 days of age, the female tendency to stay on one of the two modules compared to male and mixed groups (65.9%, 60.9% and 56.8%; P=0.01) was again noticed. Females group spent less time moving (1.11% in female pens, 1.40% in male pens, 1.33% in mixed pens; P<0.01), running (0.14%, 0.23%, 0.21%; P=0.04), allo-grooming (1.49%, 2.08%, 2.02%; P=0.02) and sniffing (1.03%, 1.91%, 1.44%; P<0.01). At 69 days of age, the effect of gender composition on budget timeaugmented: in the female pens, less time was devoted in moving (0.50%, 1.66% in male pens, 0.95% in mixed pens; P<0.001), running (0.16%, 0.48%, 0.27%, P<0.001), sniffing (0.53%, 1.62%, 1.30%; P<0.001), allo-grooming (0.88%, 1.82%, 1.17%; P<0.001), sitting (0.29%, 0.49%, 0.57%; P<0.001), stretching and digging (0.02%, 0.14%, 0.03%; P<0.01). At the open filed test, rabbits from mixed-sex pens were less active, as they crossed a lower number of squares (26.8 in mixed-sex pens vs. 38.2 in female pens vs.37.7 in male pens; P=0.06), moved for a shorter time (30.6 vs.44.9 vs. 36.7 s; P=0.05) and stood still for a longer time (59.9 vs. 45.9 vs. 34.9 s; P=0.02). During the novel-object test, the number of contacts between rabbit and object was significantly higher in pens with only males (8.15 vs. 6.97 vs. 6.51 contacts/rabbit in male, female and mixed pens; P=0.01). Hair corticosterone concentration was higher in the rabbits kept in the mixed groups (6.65 ng/g in mixed pens vs. 5.23 ng/g in female pens vs. 5.87 ng/g in male pens; P=0.02).

In conclusion, rabbits showed a clear preference for the plastic floor rather than the wire-net and even less for the steel slat. Preference differences tended to fade with the increase of age, due to the increase of space requirements. Animals reared on the most acceptable floor combination (wire-net/plastic) were also more keen on exploring the new environment, while budget time was not influenced to a great extent by the floor combinations. Rearing in mixed groups resulted in some effects on rabbit reactivity and in higher hair corticosterone level. The latter result may be correlated with differences in aggressiveness among animals or body injuries, which were not observed, however. Thus, further investigation should be done to assess the usefulness of rearing growing rabbits in single-gender groups.

On the base of the results of the present Thesis, pen housing may be a succesfull way for growing rabbits giving productive results comparable with those obtained in the current cage systems, but only if housing conditions are suitable for the animals.

Surely, rabbits reared in pens and in groups demonstrated a complete, typical-for-the-species behavioural repertory. The conditions of pen housing tested in the present Thesis, i.e. individual and functional space availability, floor type, gender composition of groups or the presence of environmental enrichment did not modify the ethogram with regards to the rate and prevalence of the main behaviours when rabbits were in a comfortable condition. Only when some factors, i.e. the wooden slatted floor, were not suitable for rabbits safe displacement, some major effects were noticed in terms of an increase of the resting time at the expense of other important activities (i.e. feeding). The same result was observed for reactivity of rabbits towards man or new environmental conditions: i.e. the experimental factors tested within pen housing systems modified rabbits reactivity to a weak extent. Once again, when housing conditions were not suitable (i.e. wooden slatted floor, tube), the rabbits reactivity was negatively affected and rabbits were more afraid to face a new situation.

From the methodological point of view and regarding the welfare indicators used, ethogram was always useful to evaluate the general condition of the animals; among reactivity tests, the tonic immobility test and the contact test were less useful to differentiate the experimental groups, whereas the reaction of the animals during the open field test was more evident and consistent with other welfare indicators. Glucocorticoids measurements had a great potentiality even if the results obtained on the different matrices need to be evaluated in a different way taking into account what kind of stress is investigated (acute or chronic stress).

In order to encourage producers to abandon the old practices and adopt alternative housing systems, security for their profit must be provided to them. This can be achieved only by associating productivity with welfare indicators during the evaluation of the new systems and verifying that both are respected.

Riassunto

L'Opinione pubblica richiede l'adozione di techniche "welfare-friendly" per l'allevamento degli animali di interesse zootecnico. L'allevamento del coniglio da carne in gabbie bicellulari (2 conigli/gabbia) oppure in gruppi piccoli (4-8 conigli/gabbia) in gabbie da riproduzione dovrà piuttosto cambiare in un sistema in colonia, in grado di migliorare il benessere animale mantenendo però le prestazioni produttive.Un sistema con gruppi di dimensioni elevate permetterebbe maggiori relazioni sociali, sulla qualità delle quali potrebbero avere un certo effetto la composizione per sessi e l'età di macellazione. Il passaggio dai sistemi convenzionali di gabbia a quelli nuovi e alternativi non potrebbe essere tuttavia possibile senza una valutazione delle conseguenze su prestazioni produttive e benessere animale.

Per raggiungere questo obiettivo generale, sono stati realizzati quattro esperimenti di seguito riassunti:

Esperimento No. 1–*Benessere e produttività di conigli da carne allevati in gabbie bicellulari e recinti collettivi*

Il presente studio ha inteso valutare prestazioni produttive, comportamento, reattività e livelli di stress di conigli in gabbie bicellulari (2 conigli/gabbia) rispetto a conigli allevati in recinti collettivi, privi di copertura e con pavimento in grigliato di legno (sezione dei listelli: 8 × 8 cm; distanza fra i listelli: 3 cm). Inoltre, entro recitni collettivi, è stato valuatato l'effetto delle dimensioni (small vs. large) e della densità di allevamento (12 vs. 16 animali/m^2) degli stessi recinti. Quattrocentocinquantasei conigli da carne (Hyplus crossbred) sono stati allevati dallo svezzamento (35 d di età) alla macellazione (77 d) in 40 gabbie bicellulari commerciali (28 × 40 × 28 cm; 2 conigli/gabbia; 18 animali/m^2); 4 recinti colletivi small (1.40 × 1.20 × 1.05 m-altezza) a bassa densità (20 conigli/recinto; 12 animali/m^2); 4 recinti collettivi small ad alta densità (27 conigi/recinto; 16 animali/m^2); 2 recinti collettivi large (1.40 × 2.40 × 1.05 m-altezza) a bassa densità (40 conigli/recinto; 12 animali/m^2) e 2 recinti collettivi ad alta densità (54 conigli/gabbia; 16 animali/m^2).

L'allevamento dei conigli nei recinti collettivi ha diminuito il peso vivo finale (2839 vs. 2655 g; P<0.01), l'accrescimento giornaliero (-10%; P<0.01), e il consumo alimentare giornaliero (-11%; P=0.001), rispetto ai conigli tenuti nelle gabbie bicellulari, senza influenzare l'indice di conversione. I conigli mantenuti nei recinti

collettivi hanno dedicato una quota maggiore del loro tempo a riposarsi rispetto a quelli nelle gabbie bicellulari (82.1 vs. 77.6% del tempo di osservazione; $P<0.01$), ad alimentarsi (7.8 vs. 10.9%; $P<0.01$), ad allo-grooming (0.65 vs. 1.58%; $P<0.001$) ed a muoversi (0.81 vs. 0.35%; $P<0.001$).

La percentuale dei conigli sensibili al test di immobilità tonica è risultata inferiore nei conigli allevati in gruppo rispetto a quelli nelle gabbie bicellulari (76.6 vs. 93.8%; $P=0.03$). Durante il test di open field, i conigli dei recinti collettivi sono stati meno in movimento (40.4 vs. 57.2 s; $P<0.001$) e hanno esplorato in maniera cauta l'arena per un tempo minore (345 vs. 371 s; $P<0.001$) rispetto a quelli delle gabbie bicellulari che hanno speso più tempo in fermo vigile (82.1 vs. 38.7 s; $P<0.001$).

I conigli nei recinti collettivi hanno presentato mediamente una maggior livello di corticosterone nel pelo (15.7 vs. 6.7 ng/g per i conigli in gabbie bicellulari; $P<0.001$).

Entro collettivi, non è stato osservato nessun effetto della densità sul comprtamento; mentre aumentare le dimensioni del recinto si è numericamente diminuito il tempo dedicato dai conigli ad alimentarsi (8.30 vs. 6.55%), a self-grooming (6.39 vs. 5.47%) ed a riposarsi (81.1 vs. 84.1%).

In conclusione, i conigli allevati in recinti in colonia hanno mostrato un repertorio comportamentale più completo, pur avendo dedicato più tempo al riposo; sono risultati meno spaventati in relazione all'uomo anche se più timorosi quando posti in una nuova situazione ambientale rispetto ai conigli in gabbie bicellulari. Nelle condizioni del presente esperimento, la dimensione dei recinti e la densità di allevamento entro i sistemi collettivi hanno avuto un effetto debole sul comportamento, il livello di paura e la condizione di stress dei conigli da carne.

Esperimento No. 2–*Effetto di pavimentazione, densità di allevamento, età di macellazione e sesso su benessere e produttività di conigli da carne allevati in recinti collettivi*

A 34 gg di età, 376 conigli di entrambi i sessi sono stati sistemati in 16 recinti (0.12 × 0.14 × 1.05 m-altezza; 1.68 m^2) per l'allevamento in gruppo e secondo uno schema fattoriale 2×2×2 con due tipi di pavimento (grigliato di legno vs. grigliato di plastica), due densità di allevamento (12 vs. 16 animali/m^2), e due età di macellazione (76 vs. 83 d). Il pavimento in grigliato di legno aveva sezione dei listelli: 8 × 8 cm e distanza fra i listelli: 3 cm; mentre il pavimento di plastica aveva fessure di 7 cm di lunghezza e 1 cm di larghezza, intervallate da uno spazio pieno di 0.7 cm. I conigli sono stati controllati per prestazioni produttive, comportamento, reattività e livello di stress.

La presenza di una pavimentazione in legno ha determinato una riduzione del peso di macellazione rispetto alla presenza di una pavimentazione in plastica (2795 vs. 2567 g; P<0.001). I conigli allevati nei gabbioni con pavimentazione di plastica hanno dedicato meno tempo alle seguenti attività: allo-grooming (0.18 vs. 0,25%), correre (0.01 vs. 0 07%), mordere (0.06 vs. 0.14%) e graffiare (0.03 vs. 0.06%) il recinto e hanno realizzato meno salti (0.01 vs. 0.07) rispetto ai conigli tenuti sul pavimento di plastica (0.001<P<0.10); inoltre, hanno speso meno tempo riposando con il corpo raccolto (27.1 vs. 31.9%; P<0.001). La reattività nel test di immobilità tonica è risultata simile in entrambi i gruppi. Durante il test di open field i conigli dei recinti con pavimento di legno hanno mostrato una minore disponibilità ad entrare spontaneamente nell'arena (60.0 vs. 72.5%; P=0.09) e sono risultati meno attivi durante il test, muovendosi meno (26.0 vs. 31.2 s; P=0.03), attraversando la parte centrale dell'arena per un numero inferiore di volte (0.48 vs. 2.27; P<0.001), dedicando meno tempo a grooming (1.94 vs. 4.25 s; P<0.01) e assumendo meno frequentemente la posizione di allerta (0.96 vs. 2.32; P<0.01). Durante i test di reattività verso un nuovo oggetto o verso il contatto con l'uomo, i conigli dei recinti con pavimento di legno hanno mostrato meno interesse verso l'oggetto o verso l'uomo (numero di contatti: 50.3 vs. 87.2% e conigli in contatto: 24.0 vs. 48.1%, rispettivamente per i due test; P<0.01). I valori di corticosterone misurati nelle feci sono risultati maggiori nei conigli dei recinti con pavimento in legno rispetto a quelli su plastica (41.7 vs. 37.8 ng/g; P=0.06).

L'aumento della densità di allevamento da 12 a 16 conigli/m^2 ha provocato una riduzione dell'accrescimento (40.2 vs. 38.7 g/d; P<0.01), del consumo di alimento (119 vs. 123 g/d; P<0.01) e del peso vivo finale (2725 vs. 2637 g; P<0.01). I conigli allevati alla densità maggiore hanno dedicato più tempo al riposo (66.6 vs. 68.9%) e meno tempo a self-grooming (16.1 vs. 14.9%) e annusare (4.49 vs. 4.11%) all'interno del recinto (0.001<P<0.06). Inoltre, è diminuita la durata dell'immobilità durante il test (68.2 vs. 49.0 s; P<0.01) e la percentuale di conigli entrati spontaneamente nell'arena nel test di open field (58.8 vs. 73.8%; P=0.04). La risposta dei conigli al test del nuovo oggetto è risultata simile; mentre durante il test di contatto con l'uomo i conigli allevati alla densità maggiore hanno mostrato una tendenza ad un maggiore contatto con l'operatore (conigli in contatto: 41.3 vs. 28.0%; P=0.08).

All’aumentare dell’età di macellazione (da 76 a 83 giorni) sono aumentati il peso vivo finale (2574 vs. 2788 g) e il consumo di alimento (119 vs. 123 g/d) ($P<0.05$); mentre, è diminuito l’accrescimento (40.0 vs. 38.6 g/d; $P=0.01$).

L’aumento dell’età degli animali al momento della registrazione ha significantivamente modificato il budget time degli animali: è diminuito il tempo dedicato a mangiare e bere (11.0 a 6.94% e 1.81 a 1.46%; $P<0.001$, rispettivamente), self- e allo-grooming (16.7 a 14.3% e 0.27 a 0.16%; $P<0.01$, rispettivamente), correre e muoversi (0.06 a 0.01% e 1.61 a 1.20%; $P<0.01$, rispettivamente) e annusare (5.49 a 3.36%; $P<0.001$), mentre è aumentato il tempo dedicato al riposo (63.0 a 72.8%; $P<0.001$).Con l’aumento dell’età, i conigli sono risultati meno attivi durante il test di open field (spostamenti totali: da 38.0 a 33.9;esplorazione: da 380 a 365 s; corsa: da 12.0 a 3.27 s; grooming: da 4.42 a 1.87 s; $P<0.001$) e hanno mostrato meno durata dell’immobilità durante il test di immobilità tonica (63.7 a 52.4 s; $P<0.001$). Si sono ridotti i contatti con l’oggetto (numero di contatti: 78.1 a 59.4%) e con l’operatore (conigli in contatto: 43.5 a 26.5%) ($P<0.001$) nei corrispondenti test del nuovo oggetto e del contatto.

La percentuale dei conigli con lesioni evidenti provocate da aggressioni fra gli animali è stato influenzato dalla densità di allevamento (8.2 vs. 26.2% nei conigli allevati a 12 vs. 16 animali/m^2; $P<0,001$), l’età di macellazione (15.0 vs. 22.2% nei conigli macellati a 76 d vs. qualli macellati a 83 d; $P=0.07$) e il sesso (11.3 vs. 25.8% nelle femmine vs. i maschi; $P<0.001$).

In conlusione, la produttività dei conigli allevati in recinti è stata in gran parte determinata dal tipo di pavimentazione e meno dalla densità di allevamento. La pavimentazione di plastica è risultata più comfortevole per i conigli, producendo così una riduzione del livello generale di paura e stress. La diversa incidenza di lesioni in funzione dei fattori sperimentali merita ulteriori indagini.

Esperimento No. 3– *Effetto del tipo di arricchimento ambientale e dell’età di macellazione su benessere e produttività di conigli da carne allevati in colonia*

Lo scopo del presente studio era valutare l’effetto di arricchimento e dell’età di macellazione su benessere e produttività di 504 conigli allevati da 33 di età fino alla macellazione in 16 recinti collettivi (0.12 × 0.14 × 1.05 m-altezza; 1.68 m^2) forniti di pavimentazione in grigliato di plastica (fessure di lunghezza: 7 cm e larghezza 1 cm intervallate da spazio pieno di 0.7 cm). Metà dei recinti erano attrezzata o meno con una piattaforma (50 × 120 cm; 30 cm sopra il pavimento di plastica) fatta dallo stesso materiale in grigliato di plastica ed, entro ogni gruppo, metà dei recinti era inoltre attrezzatao meno con un tubo in plastica da edilizia (ϕ 20 cm; lunghezza: 50 cm). Infine, i conigli sono stati macellati a 68 giorni di età (8 recinti) o 75 giorni (8 recinti).

Le prestazioni produttive non sono state influenzate dalla presenza o meno della piattaforma, mentre la percentuale di animali che presentavano ferite ai genitali prima della macellazione è stata significativamente superiore nei conigli allevati nai recinti con piattaforma piuttosto che in quelli senza (20.6 vs. 11.7%; P<0.01). La presenza del tubo ha negativamente influenzato l'accrescimento (39.7 vs. 43.4 g/d per presenza vs. assenza del tubo; P<0.001) e il peso vivo finale (2467 vs. 2600 g; P<0.01).

Il tubo non ha influenzato il comportamento degli animali nel corso delle 24 ore, mentre il budget time è risultato diverso in presenza della piattaforma: i conigli nei recinti con piattaforma hanno dedicato più tempo a riposo con il corpo disteso (33.7 vs. 31.4%), alimentazione (11.3 vs. 8.49%) e manifestazione di comportamenti specie-specifici (leccare e mordere elementi: 0.37 vs. 0.21%, allerte: 0.,13 vs. 0.06%). Gli elementi di arrichimento non hanno modificato la risposta dei conigli al test di open field o di immobilità tonica, mentre la presenza del tubo ha determinato un aumento del livello di corticosterone nel pelo (8.06 vs. 6.74 ng/g; P=0.02).

L'aumento dell'età di macellazione ha aumentato il peso vivo finale (2467 vs. 2600 g; P<0.01). Con l'avanzare dell'età degli animali, è stato rilevato un tendenziale aumento delle lesioni ai genitali (15.6 vs. 18.0%; P=0.08). Gli animali più maturi hanno dedicato più tempo a riposo con il corpo raccolto (33.2 vs. 27.8%), self-grooming (17.7 vs. 13.4%) e sniffing (5.24 vs. 3.71%) e meno tempo a comportamenti specie-specifici (0.98 vs. 1.23%) (P<0.001).

Durante il test di open field, i conigli di età maggiore hanno attraversato meno volte il riquardo centrale (3.41 vs. 4.25) e speso più tempo per esplorazione (534 vs. 511 s), corsa (7.66 vs. 5.48 s), grooming (6.99 vs. 6.75 s) e mordere elementi dell'arena (11.7 vs. 2.87 s) (P<0.05). All'aumentare dell'età anche aumentata la concentrazione di corticosterone misurata nel pelo (8.73 vs. 6.07 ng/g; P<0.001).

I conigli maschi hanno mostrato un minore accrescimento rispetto alle femmine, di modo che alla fine del periodo sperimentale i conigli maschi sono risultati anche più leggeri (2508 vs. 2559 g; P=0.02). La percentuale di conigli che presentavano lesioni a livello dei genitali è risultata maggiore nei maschi (29.3 vs. 4.74%; P<0.001). Durante il test di open field le femmine sono risultate meno attive (tempo di latenza:

24.0 vs. 19.7 s; fermo vigile: 57.7 vs. 45.6 s; grooming: 6.03 vs. 7.84 s; mordere elementi: 5.16 vs. 6.51 s) rispetto ai maschi (P<0.001).

In conclusione, l'impiego di recinti attrezzati con piattaforme permette di aumentare il numero di conigli senza effetti negativi, mentre la presenza di un tubo per arricchimento ambientale compromette la produttività e il benessere degli animali. All'aumentare dell'età di macellazione, alcuni risultati negativi sono stati esaltati, accompagnandosi ad un aumento dell'aggressività fra gli animali. Infine, resta da chiarire se l'allevamento in gruppi separati per sesso possa ridurre l'aggressività e la presenza di lesioni sugli animali allevati in gruppi di elevate dimensioni.

Esperimento No.4 – *Effetto della composizione per sesso e del tipo di pavimentazione su benessere e prestazioni produttive in conigli da carne allevati in recinti in colonia*

Un totale di 288 conigli, allevati in 18 recinti collettivi con 16 animali per recinto dallo svezzamento alla macellazione (33-74 giorni di età), sono stati controllati per prestazioni produttive, comportamento, reattività e stress. Ciascuno dei recinti era costituito dall'unione di due moduli (64 x 78 x 110 cm; 0.5 m^2/modulo) collegati tra loro mediante un'apertura (23 × 25 cm) sulla parete in comune. I due moduli ciascun recinto presentavano due pavimenti diversi, secondo tre diverse combinazioni: acciao/plastica vs. acciaio/ferro vs. ferro/plastica. Inoltre, i recinti potevano presentare gruppi di diversa composizione per sesso (solo femmine vs. solo maschi vs. misti).

Le prestazioni produttive non sono state influenzate dai fattori sperimentali, ma i conigli hanno mostrato una preferenza diversa per le diverse pavimentazioni presenti nei due moduli dello stesso recinto: mediamente, sono stati trovati 5.57 conigli nei moduli con pavimentazione in grigliato di acciaio, 7.79 conigli nei moduli con pavimentazione in rete di ferro e 10.64 conigli nei moduli con pavimentazione in grigliato di plastica (P<0.001). Solo a partire dai 59 giorni di età la distribuzione degli animali è risultata piu simile nei due moduli dello stesso recinto a causa dell'aumento delle dimensioni degli animali e della riduzione dello spazio disponibile.

L'analisi dell'etogramma (a 42, 56 e 69 giorni di età) in recinti con diversi dipi di pavimentazione ha confermato la preferenza degli animali per il modulo con il pavimento di plastica (quando era preserente) piuttosto che quelli con pavimento di ferro o di acciaio. Durante il test di open field, i conigli allevati nei moduli con pavimentazione ferro/plastica hanno mostrato un comportamento più attivo di quelli allevati sugli altri pavimenti: sono entrati spontaneamente dentro l'arena (64.7%, 30.6%, 33.3% dei conigli nei recinti con ferro/plastica, acciaio/ferro e acciaio/plastica, rispettivamente; P=0.01), hanno attraversato un maggior numero di quadrati (42.4, 30.4, 30.1; P=0.03), corso più lungo (3.59 s, 1.72 s, 1.78 s; P=0.03) e effettuato un maggior numero di salti (0.47, 0.19, 0.25; P=0.05).

A 42 giorni di età, le femmine hanno dimostrato una chiara preferenza per stazionare più su uno stesso modulo (percentuale di conigli presenti nell'uno dei due moduli: 77.5%), tendendo a rimanere raggruppate rispetto ai maschi (74.8%) e ai gruppi misti (71.7%) (P=0.03); il numero dei salti è risultato significamente superiore (P=0.05) nei recinti con sole femmine (1.31) piuttosto che in quelli con soli maschi (1.01) o gruppi misti (1.04). Nei recinti con soli maschi i conigli hanno speso meno tempo nel movimento (1.58% nei recinti con soli maschi, 1.90% nei recinti con sole femmine, 1.84% nei recinti con gruppi misti; P=0.04). A 56 giorni di età, la tendenza al raggurppamento in uno solo modulo nei recinti di sole femmine è stata mantenuta rispetto ai gruppi di soli maschi e ai gruppi misti (65.9%, 60.9% e 56.8%; P=0.01). Nei recinti con sole femmine, i conigli hanno dedicato meno tempo al movimento (1.11% nei recinti con sole femmine, 1.40% nei recinti con soli maschi, 1.33% nei recinti con gruppi misti; P<0.01), alla corsa (0.14%, 0.23%, 0.21%; P=0.04), all'allo-grooming (1.49%, 2.08%, 2.02%; P=0.02) e ad annusare (1.03%, 1.91%, 1.44%; P<0.01). A 69 giorni di età, nei recinti con sole femmine, gli animali hanno speso meno tempo nel movimento (0.50%, 1.66% nei recinti con soli maschi, 0.95% nei recinti con gruppi misti; P<0.001), nella corsa (0.16%, 0.48%, 0.27%, P<0.001), nell'annusare (0.53%, 1.62%, 1.30%; P<0.001), nell'allo-grooming (0.88%, 1.82%, 1.17%; P<0.001), nella posizione "seduta" (0.29%, 0.49%, 0.57%; P<0.001), nello stirarsi e nello scavare (0.02%, 0.14%, 0.03%; P<0.01).

Durante il test dell'open field, i conigli provenienti dai recinti con i gruppi misti sono risultati meno attivi, avendo attraversato un numero minore di riquadri (26.8 nei conigli dei recinti con gruppi misti vs. 38.2 nei conigli dei recinti con sole femmine vs. 37.7 nei conigli dei recinti con soli maschi; P=0.06), avendo speso meno tempo nel movimento (30.6 vs. 44.9 vs. 36.7 s; P=0.05) e più tempo in fermo vigile (59.9 vs. 45.9 vs. 34.9 s; P=0.02). Durante il test del nuovo oggetto, il numero di contatti fra conigli e oggetto è risultato significativamente superiore nei recinti con soli maschi (8.15 vs. 6.97 vs. 6.51 contatti per coniglio nei recinti con maschi, femmine, gruppi misti; P=0.01). La concentrazione di corticosterone misurata nel pelo è risultata superiore nei conigli mantenuti in gruppi misti (6.65 ng/g nei conigli dei recinti con gruppi misti vs. 5.23 ng/g nei conigli dei recinti con femmine vs. 5.87 ng/g nei conigli dei recinti con maschi; P=0.02).

In conclusione, i conigli hanno mostrato una netta preferenza per il pavimento di plastica rispetto a quello di ferro e ancora meno per quello di acciao. Le differenze di preferenza sono progressivamente scomparse all'aumentare dell'età degli animali a causa della riduzione dello spazio disponibile. Gli animali allevati sulla combinazione di pavimentazione più gradita (ferro/plastica) hanno mostrato una maggiore attitudine all'esplorazione di un nuovo ambiente, mentre il budget time non è stato influenzato in maniera rilevante. I conigli allevati in gruppi a sesso misto hanno mostrato alcune differenze di reattività rispetto ai gruppi dello stesso sesso, oltre che un maggiore livello di corticosterone nel pelo. Questo ultimo risultato potrebbe essere correlato con una maggiore aggressività fra gli animali nei gruppi misti, che però non è stata confermata da differenze nella presenza di lesioni sul corpo degli animali. Resta dunque da calutare l'opportunità di un allevamento a sessi separati.

Sulla base dei risultati ottenuti dalla Tesi presente, è possibile affermare che l'allevamento dei conigli da carne in recinti collettivi e con dimensioni del gruppo anche relativamente elevate può dare risultati produttivi paragonabili a quelli ottenuti nelle gabbie tradizionali, purché non ci siano condizioni in grado di causare un disagio agli animali.

I conigli allevati in gruppo in recinti mostrano un repertorio comportamentale più completo e tipico per la specie. Le condizioni di stabulazione nei recinti testate nella presente Tesi, ossia la disponibilità di spazio individuale e funzionale, il tipo di pavimentazione, la composizione per sesso dei gruppi, oppure la presenza di arricchimento ambientale, non hanno modificato il comportamento dei conigli in termini di incidenza o prevalenza dei principali comportamenti. Solo quando alcuni fattori, es. la pavimentazione con listelli di legno, non sono risultati idonei per una agevole e sicura attività motoria dei conigli, sono stati osservati alcune variazioni come un aumento del tempo dedicato al riposo a scapito di altre attività importanti (alimentazione). In maniera simile, i fattori sperimentali testati entro i sistemi di stabulazione in recinti hanno modificato la reattività dei conigli in maniera debole. Solo quando le condizioni di stabulazione non sono sono risultate idonee adatti (pavimentazione di legno, tubo), la reattività dei conigli è stata influenzata negativamente e i conigli hanno mostrato più paura di fronte ad una nuova situazione.

Dal punto di vista metodologico e per quanto riguarda gli indicatori di benessere usati, l'etogramma è stato sempre utile per valutare le condizioni generali degli animali; fra i vari test di reattività, il test di immobilità tonica e il test di contatto sono apparsi meno utili per differenziare i fattori sperimentali, mentre la reazione degli animali durante il test di open field è stata più evidente e coerente con altri indicatori di benessere. Le misure dei livelli di glucocorticoidi hanno mostrato una grande potenzialità, anche se i risultati ottenuti dalle vari matrici devono essere valutati in modo diverso in funzione del tipo di stress che si intende misurare (stress acuto o cronico).

Affinché i produttori possano abbandonare i sistemi di allevamento tradizionali e orientarsi verso sistemi alternativi, occorre fornire loro tutte le informazioni necessarie per garantire la produttività e i risultati economici. Ciò puo essere solo conseguito solo effettuando la valutazione dei nuovi sistemi sulla base di indicatori di produttività e indicatori di benessere.

Introduction

Why animal welfare?

The revolution on the animal husbandry followed the 2nd World War led to great changes to the sector. Mainly after the second half of the 20th Century, meat production flourished and the development of intensive husbandry systems in order to satisfy the increased demands of the more industrialized societies was a fact (Fraser et al., 2008). Farmers, in order to elevate their profits, were giving priority in keeping animals in restricted space and usually in barren cages or pens. Additionally, a lot of the procedures that had been taking place traditionally by farmers were now mechanised (feeding, milking, collection of eggs, etc.), breeding of animals focused on the optimization of productivity, whereas producing with the lowest possible costs was the main goal (Broberg, 2006). On the one hand, farm animals had more possibilities for easier access to food and water, to controlled environmental circumstances adapted to their needs, to medical care and to be protected by their natural predators. But, on the other hand, animals were living, very often, in cages of small dimensions and at high stocking densities, the chances to express their natural behaviours were limited.

Consequently, an outburst of public belief happened and the issue of animal welfare became increasingly prominent. Changes happened the twentieth century could have been triggered public reaction: people started interacting with animals for company and not only for farming, the diffusion of television brought people more close with animal species unknown before, etc. (Fraser, 2005). More and more consumers started concerning the way farm animals were treated before and during their slaughter and wondering about the quality of food products they were consuming, leading to the definition of the farm animal welfare science during the decade of ‘60 (Blokhuis et al., 2008). Cornerstone of that public outbreak was Harrison Ruth’s book entitled ‘Animal machines’ and released in 1964. In this book, Harrison strongly criticized the intensive rearing methods, especially for broilers, veal and pigs and had such a great impact, especially on British public, that in 1965 the government established the Brambell Committee to investigate thoroughly the way in which rearing was taking place and to suggest ways for improvement (Broberg, 2006).

Animal welfare definition

To evaluate how current production practices affect welfare of farm animals, it is necessary to define what *Animal Welfare* exactly means.

Farm animals have needs that should be fulfilled during rearing. According to Fraser and Broom (1997), "the general term *need* is used to refer to a deficiency in an animal which can be remedied by obtaining a particular resource or responding to a particular environmental or bodily stimulus". Of course, the needs are not similar among the different species of animals and according to Carenzi and Verga (2009) they may be divided in two categories:

- Environmental needs (housing systems and management);
- Physiological and behavioural needs (expressing the biological functions and the behavioural patterns of the species).

Understanding animal needs have been the basis for the formulation of 'The five freedoms', firstly by 'The Brambell Report' (1965) and then revised in 1993 and again in 2011 by FAWC (Farm Animal Welfare Council) as follows:

1. *Freedom from Hunger and Thirst* - by ready access to fresh water and a diet to maintain full health and vigour;
2. *Freedom from Discomfort* - by providing an appropriate environment including a shelter and a comfortable resting area;
3. *Freedom from Pain, Injury or Disease* - by prevention or rapid diagnosis and treatment;
4. *Freedom to Express Normal Behaviour* - by providing sufficient space, proper facilities and company of the animal's own kind;
5. *Freedom from Fear and Distress* - by ensuring conditions and treatment which avoid mental suffering (FAWC, 2011).

Nevertheless, because of the multi-faced characteristics of the *animal welfare* term, an absolute definition is difficult to be attributed. According to Carenzi and Verga (2009), "this word must reflect a clear concept, which can be scientifically assessed and which can be included in laws". In fact, until now various definitions have been proposed, as follows.

In 1946, WHO (World Health Organization) association defined welfare as "a state of complete physical, mental and social well being and not merely the absence of disease or infirmity".

In 1965, Brambell Report stated that "welfare is a wide term that embraces both the physical and mental well-being of the animal. Any attempt to evaluate welfare, therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and functions and also from their behaviour".

In 1976, Hughes mentioned that "welfare is a state of complete mental and physical health, where the animal is in harmony with its environment".

In 1986, Broom reported that "the welfare of an animal is its state as regards its attempts to cope with its environment".

In 1993, Broom and Johnson presented animal welfare as "a state where the animal adapts to its environment without overtaxing its capacities, while stereotypies, modification of adrenals' activity etc. are considered as signs of the environment overtaxing the animal".

In 1996, Stafleu defined that welfare is "a state where the animal can fulfil its needs and wants" (Veissier and Forkman, 2008).

In 2011, OIE (World Organisation for Animal Health) in the 'Terrestrial Animal Health Code' (OIE, 2011) defined animal welfare as follows: "Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress".

Moral issues

The perception about welfare of animals kept for production varies a lot among people. Western European societies may have different ethical concerns from those in the Middle-East or in North America, Far East or Africa. Also within the same society, different points of view may be met, giving gravity to different aspects of welfare. For instance, a production scientist may approach animal welfare from the productivity point of view, while a veterinarian from the health point of view or an ethologist may focus on the possibility animals have to express their species-specific behavioural repertoire (Duncan, 2005). Also, it has to do with people's income and their educational level. In fact, people who may have a low income but they are educated, seem to be ready to spend more for a "welfare-friendly" product rather than to buy from a market that supports farmers who treat animals with cruelty (Broom, 2006a). Religion and beliefs play an important role. Hence, Judeo-Christian and Islamic religions support that humans are above all and animals have been created to serve them (British Humanist Association, 2007); while, Buddhists are more keen on respecting the interests of animals (Croney and Millman, 2007). Others believe that it is not inappropriate to treat animals with harshness, while they have no soul or, alternatively, treating them with respect to the fact that they are living beings contributes in becoming better persons (British Humanist Association, 2007).

According to Dawkins (1998), it is that animals have "a mental capacity for experiencing pain, thirst, boredom and other mental states that gives animal welfare its moral edge". The key-issue about animal welfare is the question: do animals are sentient beings?. A sentient being is the one that has some ability to evaluate the actions of others in relation to itself and third parties, to remember some of its own actions and their consequences, to assess risk, to have some feelings and to have some degree of awareness (Broom, 2006b). Awareness is defined as a state in which complex brain analysis is used to process sensory stimuli or constructs based on memory (Broom, 1998). In 1997 the European Union, with the protocol on protection and welfare of animals of the Treaty of Amsterdam stated that "desiring to ensure improved protection and respect for the welfare of animals as sentient beings" (Treaty of Amsterdam, 1997). To date, thanks to Lisbon Treaty, animals are recognized as sentient beings (Lisbon Treaty, 2009).

Farm animal welfare and citizens' opinion

Beyond question, public opinion has a great force and may, drastically, influence the course of every effort for improvement regarding farm animal welfare in a society. Awareness has been spreaded in all over the world and a lot of studies have been conducted to estimate this public alertness. In Spain, a survey on 3978 people showed a high level of concern: women were more sensitized than men (85 vs. 74%, respectively) and younger (or of medium age) than older people (>78 vs. 71%, respectively) (María, 2006). When direct and indirect questions were asked to US householders, it was revealed that they were actually less concerned than they were stating (Lusk and Norwood, 2009). In a recent survey conducted in 29 provinces of China, almost the one third of the respondents had not previously heard about animal welfare; however, the 44% of the sample negatively criticized the current husbandry practises, whereas need for further informing by government was the message of the study (You et al., 2014).

Looking at it from another standpoint, in the legislative study of the Food and Agriculture Organization of the United Nations-FAO (FAO, 2010), it is stated "Public Awareness as a Central Legislative Goal". Indeed, plenty of countries when established laws about the protection of animals had public information and alertness as one of their main objectives. For instance: Japan's Act of Welfare and Management of Animals (1973), Israel's Animal Protection Law (1994), Peruvian Law on Protection of Domestic Animals and Wild Animals Kept in Captivity (2004), Austrian Animal Protection Act (2005), The Korean Animal Protection Law (2007) and others (FAO, 2010).

Focusing in the European Union, a survey conducted on European countries (Hungary, Italy, France, the United Kingdom, the Netherlands, Norway and Sweden) in 2005 showed that a high percentage of people admitted that farm animal welfare is very important for them, with Italy at the highest level (75%) and Netherlands at the lowest one (35%). In the same study, Italians were the most worried concerning transportation and slaughter; whereas the Norwegian and Sweden were the less worried about pig, poultry, dairy cows welfare, slaughter and transportation. Lastly, however Norwegian, Swedish and Hungarian claimed that animal welfare is an essential issue for them, they seemed not to be such worried about rearing conditions of farm animals in their countries (Welfare Quality Report, 2007). In the study carried out by the European Commission on the 25 Member States and four accession and candidate countries in 2006, it was revealed that European population is highly aware of farm animal welfare. Indicatively, to the question “on a scale of 1-10 how important is it to you that the welfare of farmed animals is protected?” the 34% replied that it was of the highest importance (10/10) and only the 2% said that they did not care; with Scandinavian (Sweden, Finland and Denmark) and Mediterranean (Cyprus, Malta, Greece) countries giving high ratings (9.1 and 7.8, respectively) (Eurobarometer, 2007).

From the above mentioned, it may be assumed that farm animal welfare is an issue of significant importance and consumers believe in a correlation between welfare and quality of products they, finally, buy (Blokhuis et al., 2008; Szücs et al., 2007). In fact, rearing animals under conditions that respect welfare has, in most of the times, positive effects on the products derived from them (Blokhuis et al., 2008; Terrestrial Animal Health Code, 2011). For example, in the poultry sector, the reduction of bone breakage incidence, meat bruising, blood spots or abnormal eggshells when welfare principles were put into practiceis proved by many studies (Blokhuis et al., 2008; Faure et al., 2003; Hughes and Curtis, 1997; Jones, 1997, 2001). As a result, after the 1st January 2012, laying hens have been reared in alternative systems or enriched cages; where, drinkers, feeders, a perch, a nest and litter are available and stocking density does not exceed the 9 hens/m^2 (alternative systems) or at least 750 cm^2 of cage area per hen (enriched cages) (Council Directive 1999/74/EC).

Generally speaking, consumers’opinion has led more and more producers to re-organize their managerial systems in order to meet their concerns. In this way, introducing to their farms “welfare-friendly” techniques may become profitable for their income (Blokhuis et al., 2008; European Commission Factsheet, 2007; Sevi, 2007). Nevertheless, because of the lack of information about the subject, these advantages are not yet clearly understandable by the farmers, the legislation about supporting “welfare-friendly” products is still under development and public opinion is not strongly established (Harper and Henson, 2001). Indeed, the 58% of the European citizens would like to be better informed about the rearing methods taking place in their countries; whereas the 77% believe that further improvement of animal welfare must be done (Eurobarometer, 2007).

On the other hand, applying new systems that promote welfare during rearing (i.e. larger cages, environmental enrichment, pain prevention during routine interventions, etc.) would probably increase costs, putting a dilemma to producers. When European citizens were asked if producers should be compensated for these extra costs, the 72% replied positively. Also, welfare-friendly products should be easily traceable by consumers and this can be achieved only by official labelling of the products. In a European study regarding welfare labelling is stated that “An animal welfare labelling scheme is a certification system that certifies an animal welfare standard above existing legal standards” (European Commision, 2009). Few countries have established labelling schemes (governmental and not) that focus on animal welfare: Freedom Food (Britain, 1994), Travelife Animal Attractions Guidelines (UK), Neuland (Germany, 1988), Animal Index System (Austria), EU egg marketing legislation based on Regulations (EC) 1234/2007 and 589/2008 (organic or free-range farms). Nevertheless, in the same report it is made clear that “there is still much uncertainty about the market share of animal welfare friendly products”.

According to Veissier and Forkman, (2008), “in countries where higher national legislation for animal welfare does not exist, and where there is no significant level of public interest in animal welfare, there is a barrier to the extent to which animal welfare standards can increase. But where strong national legislation goes together with high public concern and a governance tradition of commoditizing public issues, the resulting dominant position of retailers and processors may also significantly increase competition among farmers”.

Welfare legislation

Brief historical overview

It is believed that the first known rule about animal protection has been released in the 3rd Century BC regarding the abolishment of sacrificial slaughter of animals, by Buddhist-Asoka, the King of Maghada in North India. While, in the western world the first legal attempts to defence animals were recorded in Great Britain, USA and France (Kolacz et al., 2007). In 1641, the Body of Liberties, founded by the Puritans of the Massachusetts Bay Colony stated: “No man shall exercise any Tyranny or Cruelty towards any brute” (Kolacz et al., 2007).

After the 2nd World War, the foundation of the Council of Europe in 1949 was the first “supra-national” organization that proposed measures about animal welfare, and in the 1960s it was supported that respecting animals was a mutual point combining all the European countries and it was closely associated with human dignity, whereas accordance among the countries was essential (Veissier et al., 2008). Then, the Brambell Report, in 1965, considered the cornerstone for further research on animal protection worldwidely, with its recommendations about suffering of farm animals to urge public opinion (Bozcurt et al., 2009). In 1974, it was proposed the first legislation about animal welfare, at European level, concerning slaughtering of animals: “Whereas the Community should also take action to avoid in general all forms of cruelty to animals; whereas it appears desirable, as a first step, that this action should consist in laying down conditions such as to avoid all unnecessary suffering on the part of animals when being slaughtered” (Commission Working document, 2006). In 1976, the Committee of Ministers of the Council of Europe organized the convention for the Protection of Animals kept for Farming Purposes, containing broad recommendations about housing of farm animals under intensive methods, which then was converted into an EU directive (Directive 98/58/EC) (Veissier et al., 2008).

Current situation

To date in Europe, a plethora of directives has been proposed by the European Commission concerning all farm animals. General rules about rearing have been recommended. Also, more specific ones have been instituted; i.e. about the protection of laying hens, calves and pigs, the protection of animals at the time of slaughtering or killing, the protection of animals during transport, etc. (European Commission, 2014).

The European Commission, with its communication about ‘The Community Action Plan on the Protection and Welfare of Animals 2006-2010’, aimed to ameliorate the existing condition about animal protection followed by another legislative document entitled 'The European Union Strategy for the Protection and Welfare of Animals 2012-2015' (European Commission, 2006 and 2012).

Until today, The European Commission has importantly contributed to the understanding and to the appliance of the farm animal welfare rules and legislations, defending the common good in respect with the animal rights. This activity has been supported, also, by other important European and global organizations. Indicatively: the Scientific Veterinary Committee, the Scientific Committee on Animal Health and Animal Welfare, the Group of Advisers on the Ethical Implications of Biotechnology (GAIEB), the European Food Safety Authority (EFSA), the World Organization for Animal Health (OIE) and the Food and Agricultural Organization of the United Nations (FAO) (Commission working document, 2006).

Lastly, significant contribution of the European Union in the field of animal welfare and food quality was the Welfare Quality® Project. The project started in 2004 and completed after five years. It was about a partnership of 44 institutions in European and Latin American countries. Its action focused on the developing scientifically based tools to assess animal welfare and to generate knowledge on practical strategies to improve animal welfare on farm and at slaughter. To do this, the interest felt on animal-based measures (animals behaviour, fear, physical condition etc.), as they show the "outcome of the interaction between the animal and its environment (housing design and management)". Throughout this Project, research was focused on three economically important species and their products: cattle (beef and dairy), pigs, and poultry (broiler chickens and laying hens) during their productive life, e.g. on farm, on transport (measures determined by examining the animals on arrival at the slaughterhouse) and at slaughter (Welfare Quality, 2009).

Focusing on the farm rabbit sector, the European Food Safety Authority (EFSA) has delivered the only, until now, relevant legal document: "The Impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits" (EFSA, 2005). In this, EFSA made recommendations about housing characteristics (floor types, cages height, group size, stocking density, etc.) for meat and reproductive rabbits under intensive rearing systems. Apart from EFSA's recommendations, which are the only official common guidelines within European Union, many European countries have been adopted their own national legislation about rabbit welfare. In United Kingdom legislation forms about farm animal welfare and specifically for rabbits rearing systems, have been established since 1987; whereas, the German World Rabbit Science Association gave few recommendations about space requirements for intensive rabbitries in 1991. Also in Switzerland, guidelines have been given in 1981 (Luzi et al., 2006). In Italy, in addition to the legislative document (Decreto Legislativo no. 146) released in 2001 about welfare for every species (Decreto Legisltivo, 2001), in 2014 the Ministry of Health published voluntary guidelines concerning rearing of farm rabbits (Ministero della Salute, 2014).

Welfare of farm animals - critical points under rearing

Farm animals reared intensively have to face novel challenges to which their nervous system is not used (handling, every day farm chores, etc.) and at the same time they are deprived of stimuli (not interesting surrounding) which could permit them to behave according to their species-specific patterns (Kowalska et al., 2012). In order to manage with these environmental challenges, animals use adaptive mechanisms, with behavioural and physiological responses to stress being part of them.

For these reasons, welfare of farm animals implies several aspects and “the only universally accepted conclusion is that there is no single measure of welfare” (Dawkins, 2003). Certainly, it has to do with animals feelings, but at the same time, it has to do also with their biological response to their surrounding, as well as the sanitary status.

“A central issue in the study of animal welfare has been, and continues to be, how can we objectively and scientifically assess the welfare state of an animal?” (Dawkins, 2003). Welfare assessment met recognition the period from 1970 to 1990 by using methods that have been still used (Lawrence 2008). Nevertheless, many things have been changed from those years and new additional methods developed to evaluate welfare. These methods are based on either animals behavioural recordings, fear tests or physiological body measurements and sanitary status providing a plethora of knowledge. “What is urgently needed, therefore, is a framework for bringing all the different measures of animal welfare together” (Dawkins, 2003).

Welfare evaluation

Taking, again, on hand the *Five Freedoms* (above mentioned), the following critical points of good welfare under intensive rearing of farm animals can be formed.

Freedom from hunger and thirst

This freedom is usually fulfilled when animals are reared intensively. It is obvious for producers that animals must have a balanced diet and clean water in their availability to be healthy, to get grow and to produce.

Nevertheless, the last decade restricted feeding has been proposed as an alternative way to control growth rates and digestive disorders in rabbits (Gidenne, 2012). In nature, animals do not eat constantly within a day or sometimes are obliged to pass restriction periods because of anavailability of food resources. On the other hand, feed restriction, literally, encroaches the first freedom, but in what extent it goes against welfare is still under debate.

Freedom from discomfort

Evidence of good welfare under rearing can be derived by environmental factors of the place that animals are housed. Taking under consideration the indications of the welfare legislations about housing of various species of animals, the following general conditions should be summed up:

- animals should live in a dry and clean place with housing characteristics (pen/cage size, stocking density, group size, space available/animal) that respect species-specific needs;
- the materials used to construct the cages or pens and the equipment (feeders, drinkers, etc.) with which animals are in every-day contact should not be harmful for animals health, whereas they should be easy to clean and disinfect;
- floor type should be made of material that do not hinder the movement of animals and do not provoke any lesion or pain to them;
- the microclimate (temperature, relative humidity, lighting, gas concentration) of the bulding/room in which animals are housed should respect the limits for health and performance of each species.

Freedom from Pain, Injury or Disease

According to Broom (2009), "Health refers to the state of body systems, including those in the brain, which combat pathogens, tissue damage or physiological disorder and health may be defined as an animal's state as regards its attempts to cope with pathology".

"Pain, fear and distress are unpleasant subjective states often grouped together as states of suffering. They are, also, commonly referred to as negative emotions or motivational affective states". Frustration may also be related to suffering (Duncan, 2004).

Pain may be defined as "an aversive sensation and feeling associated with actual or potential tissue damage" and, undoubtedly, plays an important role in welfare of farm animals. A lot of methods have been used in order to evaluate pain in animals (tail flick response of rats since 1941, jaw opening since 1964, etc.). Pain evaluation may be focused on behavioural observations, which differ according to the involved species and may include loud vocalizations when they are in danger or feel pain (Broom, 2001).

Under farming, various circumstances may lead to pain. For example, injuries resulting from poor farming environment, as above mentioned. Additionally, surgical interventions taking place routinely (e.g. castration, dehorning, ear-tagging, tail-docking) without applying the necessary measures to avoid pain (e.g. anaesthesia) also result in pain. Lastly, metabolic pathologies linked to growth performance may end up to pain. For instance, many studies have shown that poultry species selected for their growth performance presented skeletal deformities leading to painful moving (Duncan, 2004).

Animals should be free of injuries (skin lesions and any wounds). The injuries may result from badly designed housing systems (sharp edges, overcrowded cages, unsuitable sufaces, etc.) or from aggressions among cage-mates.

Freedom to Express Normal Behaviour

Animals have developed "behavioural strategies" allowing them to ameliorate their ability to obtain resources, escape from risks, expand their life and improve their reproductive capacity in the "evolutionary environment" (King, 2003).

Behaviour is "intentional", leaning to achieve a certain goal. Sometimes, under modern rearing systems animals do not have the opportunity to fully achieve these goals leading to excessive stress (King, 2003; Kowalska 2008).

An animal species that leaves under natural social and environmental conditions, expresses behavioural patterns (behavioural repertoire) characteristic for the species. These patterns are used to called "fixed action patterns" (FAPs) (Mattiello and Panzera, 2008).

Freedom from Fear and Distress

As mentioned above, "pain, fear and distress are unpleasant subjective states often grouped together as states of suffering" (Duncan, 2004).

The types of stimuli that may lead to *fear* are those that "are sudden and intense, novel, have been associated with danger in the animal's evolutionary history, and are associated with certain social situations" (Duncan, 2004). Fortunately, the behavioural response to fear stimuli is clearly defined. When an animal is confronted with a fearful stimulus may exhibit avoidance and move away from it or demonstrate immobility and freezing behaviour.

Distress may be defined as "a state where the animal has to devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation. Stimuli potentially leading to distress are thus more or less extreme values or levels of the various factors constituting the animal's environment" (FELASA, 1994).

Concerning *frustration*, it may be defined as the sentimental that "occurs when behaviour that is strongly motivated is prevented from occurring by some aspect of the environment" (Duncan, 2004). For instance, when hens stocked in battery cages are prevented from manifesting scratching with feet and then pecking the same area, as they normally could have done in nature (Duncan, 2004). Intense frustration may result in mental suffering.

Stress

When one or more of the points above are not fulfilled, the animals are not in a good welfare condition; instead, they are subject to different degrees of stress.

The term of stress has to do with "the behavioural, physiological and emotional status of the animal confronted with a situation that it perceives as threatening with respect to the correct functioning of its bodily or mental state" (Terlouw et al., 2008). Therefore, stress may be evaluated as behavioural or physiological adaptations to the situation faced by the animal.

The difference between the terms *stress* and *distress* is slim. Concerning animal welfare, the difference is made among stress responses that may harm the animals or not. Stress *per se* may not, necessarily, affect in a bad manner animal welfare or the latter may be influenced even if signs of stress are not obviously comprehensible; but *distress* has a negative impact on an individual's well-being. Nevertheless, this distinction is controversial (Borell, 2001).

A lot of research has been done in order to make clear how stress mechanism works in animals and in what extent may affect their physiological function. According to Dantzer (1994), the stress reactions are organized by a "complex mechanism": a cabled neuronal system (sympathetic and adrenomedullary system) responsible for a rapid transmission of the signal (a few seconds between perception of the stressor and catecholamine release) and a neuroendocrine system (pituitary-adrenal axis) responsible for slower transmission (several minutes between perception and adrenocortical response).

The *sympathetic and adrenomedullary system* is not an anatomical system but a "functional entity", which consists of the orthosympathetic branch (autonomic nervous system) and the adrenal medulla (sympathetic ganglion). When a stressful stimulus triggers this system, a catecholamine secretion follows, thus noradrenaline is released at nerve endings and adrenaline into the bloodstream by the adrenal medulla. This stimulation has a number of effects on the physiological systems. In details, the effects on the cardiovascular system are focused on the increase in the rate and force of cardiac contractions, on the increase in arterial pressure and on the redistribution of visceral blood towards muscles and brain (Dantzer, 1994). All these are part of body's adaptation in order to demonstrate a proactive coping response to the stressor.

According to Koolhas et al. (1997), "coping can be defined as the behavioural and physiological processes necessary to reach homeostasis", thus "the effort of the body to maintain a stable internal environment to challenges from widely variable environments" (Borell, 2001). Research on the subject has shown that the proactive animals (react to the stressors with aggressive and locomotion behaviours) seem to be commanded by the sympathetic nervous system, while the reactive animals (less prone to face the stressors and prefer to escape them through immobility) seem to be commanded by the parasympathetic nervous system (responsible for energy conservation during stress) (Koolhas et al., 1999).

In the organism, catecholamines have great metabolic effects, with glycogenolysis and lipolysis providing energy for actions. Additionally and regarding the renal system, catecholamines provoke a vasoconstriction of autonomic origin by diminishing glomerular filtration of sodium ions and at the same time triggering the rennin-angiotensin-aldosterone system. Furthermore, apart from the effects of the catecholamines' release on various physiological systems, effects have been observed also in the metabolism (synthesis and catabolism) of the neurohormones themselves. Hence, the activity of the enzymes taking part in the synthetic procedures of the catecholamines increases. Simultaneously, a decrease in the activity of enzymes that takes place in the catabolism of the catecholamines is detected (Dantzer, 1994).

As far as the *pituitary-adrenal axis* or else hypothalamic-pituitary-adrenocortical (HPA) axis mechanism is concerned, it is briefly described subsequently. The cerebral cortex reacts to the external stimuli by eliciting nervous signals that stimulate corticotrophin-releasing hormone (CRH) generated by neurons in the paraventicular nucleus (PVN) of the hypothalamus. The PVN collects plethora of inputs from other hypothalamic nuclei (the brain stem, the subfornical organ and the limbic system), which produce signals associated with the emotional state. The CRH is transported by the hypophyseal portal blood system to the anterior pituitary, provoking there augmentation of the synthesis and secretion of ACTH (adrenocorthicotropin hormone), β-endorphin, β-lipotropin and α-melanocyte-stimulating hormone/α-melanotropin. The ACTH ends up into the blood circulation, causing the synthesis and release of glucocorticoids (cortisol, corticosterone) in the adrenal cortex (Borell, 2001; Dantzer, 1994; Mormède et al., 2007).

The stimulation of the HPA axis varies a lot. On the one hand, its variability has to do with the periodicity of cortisol secretion, which is pulsatile and of about 90 min. On the other hand, it has to do with the diurnal cycle, "that is genetically determined and synchronized by light" (Mormède et al., 2007). The maximum level of glucocorticoids in biological levels happens at the end of the dark period, for diurnal animals, while at the end of the light period, for nocturnal animals (like rabbits). Lastly, the HPA axis is, also, altered according to environmental factors (temperature, humidity) (Mormède et al., 2007).

Cortisol or/and corticosterone secretion is increased in blood few minutes after the stressor and it is sustained for about an hour after the ending of the stressful event. However, the latitude of the response is not the same among the species. Also, the kind of glucocorticosteroids varies among species, with cortisol being the dominant in cattle, sheep, pig, mink, fox and fish, and corticosterone in birds and rodents (Mormède et al., 2007). According to Szeto et al. (2004), both cortisol and corticosterone are influenced by social stress and constitute good stress indicators for rabbits.

Glucocorticoids released under stress have a variety of effects on the organism. Firstly and regarding energy reserves, they reduce glucose capture by tissues and increase glucose synthesis from non-lipid substrates, promoting neoglycogenesis and inducing protein catabolism. All these lead to an increase in glucose, which may be further used or stored, regulating the energy balance (Dantzer, 1994).

Welfare of growing rabbits

Rabbit meat production

Since 1990, rabbit meat production has flourished; however, not homogeneously. Hence, in China the production has launched, whereas the same has not been noticed for America or Africa. In Europe, however rabbit meat production has been diminished by 15%, it still produces the 30% of rabbit meat in the world, and specifically in the Mediterranean Europe (Italy, Spain, France) (FAO, 2012).

As far as rabbit meat consumption is concerned, world average is about 0.3 kg/inhabitant, with Malta, Italy, Cyprus, Spain, France, Belgium, Luxemburg and Portugal being the major consumers (1.5 kg/year/inhabitant, on average) (FNOVI, 2010).

In Italy, rabbit production has always been an important sector of livestock. According to ISTAT (2007), rabbit farms are up to 30.000 (20% in the North, 30% in the Central and 50% in the South Italy); whereas, 170 millions of rabbits are led to slaughter every year (84% in the Nord and specifically 35% in the Veneto region) placing rabbit meat in forth place after the one of pork, beef and poultry consumption.

Nowadays, in most of the European countries fattening rabbits are housed intensively to achieve high levels of productivity. Thus, they are reared from weaning (around 4-5 weeks of age) to slaughter (around 10-12 weeks of age) in pairs (Italy, Hungary) or in small groups of 4 to 8 animals per cage, in galvanised wire-net bicellular or collective multifunctional cages, respectively, and at density of about 15 to 18 animals/m^2 (Buijs et al., 2011b; Combes et al., 2010; Di Meo et al., 2003) (Table 1).

On the one hand, under these rearing conditions rabbits are easier to be controlled and cured in case of sickness, giving satisfying productive results. On the other hand, they are not free to fully express their typical behavioural repertoire, like hopping, running, jumping, rearing on their hind legs, foraging and digging; they, frequently, exhibit stereotypic behaviour (i.e. wire-gnawing), sometimes locomotive disorders, because of limited space and high level of stress (Jordan et al., 2006; Verga, 2004).

Table 1. Characteristics of cages and stocking density used for fattening rabbits in European countries (unchanged by Trocino and Xiccato, 2006).

Country/ Type of cage	Width (cm)	Depth (cm)	Height (cm)	Total surface (cm^2)	Rabbits/cage	Individual surface (cm^2)	Stocking density (rabbits/m^2)	Average slaughter weight (kg/m^2)
France/ Belgium								
Multi-function	40	90-100	29-30	360-400	6-7	570-515	17.5-19.4	40.3-46.6
Italy/ Hungary								
Fattening in pair	28	43	35	1200	2	600	16.7	41.8-41.5
Multi-function	38	95	35	3600	5-6	720-600	13.9-16.7	34.8-45.0
Spain								
Multi-function	40	85	33	3400	7-8	485-425	20.6-23.5	45.3-51.7

In order to motivate farmers to adopt productive systems different from the existed, alternative techniques should be specified, which will be economically advantageous and they will combine rabbits welfare with satisfying productive and meat quality traits.

Unfortunately and as it has been above mentioned, the only representative official guidelines concerning housing and husbandry systems of farm rabbits are the recommendations of EFSA (2005). According to the latter, rabbits reared for their meat should be housed in collective cages with the following minimum standards: 35-40 cm of width, 75-80 cm of length, 38-40 of height, 625 cm^2 of individual surface, in groups of 7 to 9 animals and with maximum average slaughter weight not exceeding 40 kg/m^2.

Indeed, a group size until the above recommended gives satisfying productive results, of course not as much as when rabbits are housed in pairs (Figure 1). Additionally, rearing in groups of such dimensions gives animals the opportunity to express behaviours rather than resting and feeding (Verga et al., 2009). It has been noticed that with the increase of group size aggressiveness increases, especially around sexual maturity; not so much because of the size *per se*, but because one aggressive individual in a large group is enough to cause a lot of injured animals, especially when space is limited and escape vents are limited (Szendrő and Dalle Zotte, 2011).

EFSA discourages the use of deep litter as floor material considering that it favours the transmission of harmful microorganisms; so, from the hygiene point of view, it pinpoints that wire-net maybe is not so against welfare (EFSA, 2005). In fact, research has also proved that the use of deep litter reduces growth performance, worsens hygiene conditions of rabbits and obliged them to devote a lot of their time in self-grooming (Dal Bosco et al., 2002; Morisse et al., 1999; Trocino et al., 2008); whereas, interesting is the preference of rabbits for plastic floor (Princz et al., 2008a).

Cage enrichment has been positively reviewed as a way to stimulate rabbits behaviour and not only. A great variety of enrichment elements have been studied; i.e. wooden sticks or mirrors (Dalle Zotte et al., 2009a; Jones and Phillips, 2005; Luzi et al., 2003; Princz et al., 2007; Verga et al., 2004) make rabbits surroundings more interesting and urge them to interact with these, rather than performing stereotypic behaviours (Verga et al., 2009). Additionally, elevated platforms have been investigated as enrichment (Lang and Hoy, 2011; Matics et al., 2014; Postollec et al., 2008; Szendrő et al., 2012a). Rabbits may use platforms to hide below when they need to withdraw from the group or to jump above. Platforms can be also used to increase the number of animals reared at the same horizontal space available.

After EFSA’s indications, research needs further development focusing on the differences between the old systems and the “new” (collective pens), but especially on the effect of group dimension, stocking density, floor type and environmental enrichment within the collective systems on behaviour, growth performance and meat quality traits.

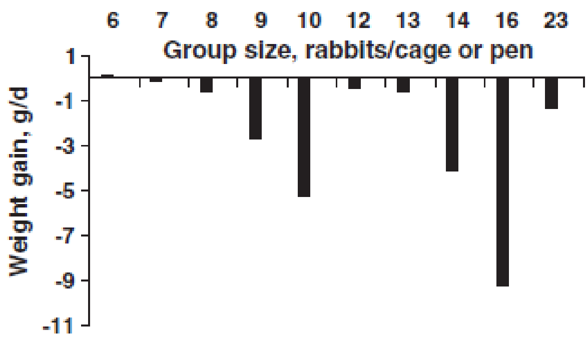


Figure 1. Effect of group size on daily weight gain. Comparison among 2-6 rabbits/cage or pen (indicated as 0 on the horizontal axis) and larger group sizes (unchanged by Szendrő and Dalle Zotte, 2011).

Measuring rabbits welfare on farm

Sanitary and productivity status

Rabbits should be kept in such a way in order their immune system be able to confront with any disease and the managerial system should be well-organized so as to prevent any disease incidence. For instance, pododermatitis from insufficient flooring conditions or coccidiosis from defective waste removal may be a problem under intensive rearing. Usage of litter may lead to hygiene problems as the falling of faeces through the mesh floor may be impeded.

Under intensive rearing, rabbits may confront with several stressful conditions (high stocking density, limited space available/animal, everyday routine chores take place at the farm, etc.) which may negatively affect their stress level. Stress impact on immune system is, partially, due to HPA axis activation, with the adrenal steroids influencing cytokine production. Hence, they suppress the action of some interleukins and tumor necrosis factor (TNF). Additionally, it has been found glucocorticoids involvement on the action of the lymphocytes. It is believed that glucocorticoids urge specific immune cells to exit the peripheral blood and access lymphatic and other tissues putting obstacles to immune system's normal action. Beyond glucocorticoids, ACTH, the second important hormone of the HPA axis, is involved in the impact of stress on the immune system, though the scenery is even cloudier in this case. The effects of ACTH releasing on immune cells depend on many factors, like its concentration and the presence and concentration of cytokines. In details and after research, it is mainly suggested that the prime line by which immune activation triggers the HPA axis is the one that involves the direct stimulation of hypothalamic CRH. Thus, the CRH is triggered by cytokines, like interleukin, tumor necrosis factor and interferon secreted by macrophages. Peripheral cytokines may reach the central nervous system (CNS) either directly using specific transporters to cross the blood-brain barrier or may trigger intra-CNS cytokine production. On the other hand, under chronic stress (chronic inflammatory states), the activation of the HPA axis is carried on by elevated hypothalamic vasopressin (VP) secretions, while CRH augmentation is noticed at the beginning of the disease. However the exact mechanism involved is not yet clear, the pure effect of CRH on immune function is believed to be the decrease of cellular immunity, including the secretion of interferon (IFN-α) (Manteuffel, 2002).

Studies have been conducted concerning the correlation between animal stress and disease susceptibility. It has been noticed that stressors suppress T- and B-lymphocyte blastogenesis, natural killer cell activity, and cytokine production, thus, interleukin-2 (IL-2) and interferon-γ (INF- γ). In fact, it is believed that the cytokines IL-1, IL-6, tumor-necrosis factor-α (TNF-α) and

INF- γ play the role of “mediators of the immunological and pathological responses to stress and infection” (Borell, 2001).

Mortality and morbidity rates and frequent controlling of animals may indicate their health status. According to the Dutch welfare regulations, the mortality of rabbits reared for meat production should be lower than 10% (Broom 1991; De Jong et al., 2011). Additionally, if rabbits live in a ‘welfare-friendly’ environment stimulates them to produce the maximum of their capacity. *In vivo* measurements of growth performance (weighing of rabbits and feed intake) give an indication about their productivity under rearing.

Behavioural evaluation

Ethogram

According to King (2003), behavioural repertoire was proposed as an indicator of good welfare. As a result, by the formulation of the ethogram information can be obtained about the conditions in which animal leaves (Mattiello and Panzera, 2008). The evaluation is species-specific and during this, behaviours that species express naturally in their behavioural repertoire should be also observed under rearing of animals. Video-recording of animals, usually, for a short period (e.g 24 hours) is used in order to analyze their behaviour. The recording is performed by using a variety of instruments, like state-of-art video-cameras and video-registrators, while the analysis of different behaviours is done, usually, by evaluating ‘behavioural units’ (seconds or times) of the different behaviours observed or even by using sophisticated software.

The behaviour of wild and domestic rabbits

Studying the behaviours of wild representatives of a species is a good starting point for welfare evaluation, because “they provide a baseline against which behaviour in captivity can be compared” (Dawikins, 1990).

The wild rabbit (*Oryctolagus cuniculus*, L.) is an important prey species for numerous carnivores (Lombardi et al., 2003). Rabbits in nature are very active and the highest part of their activities takes place during nocturnal period, beginning at dusk and decreasing until dawn, achieving in this way their camouflage. The animals manage to survive and defend themselves against predators by mimitism, escaping, good knowledge of their territory and their great reproductive capacity.

Wild rabbits are social animals which live in communal groups of one to three males and one to five females into burrows. Dominance hierarchies exist in parallel for both males and females. The territorial boundaries of each group are formulated by the dominant male setting the limits by chin gland secretions and faeces/urine excretions. As the population grows rapidly, more and more competition and aggressiveness occur within and between groups and the territorial delineation is playing an important role, with the dominants being the most aggressive ones. Females manifest aggressiveness in order to defend the breeding burrows, while males do it to prevent any new male entrance into the breeding group.

In order to construct their burrows, rabbits prefer well-drained soils, neither sandy nor rocky, with wooded or shrub areas. The burrows are mostly created by females as the reproductive period approaches (usually the first days of September until the end of December), while males participate in this construction for establishing bonds with the group. However, they may also adapt well on wetlands, creating their shelter in bushes and logs instead of digging burrows (Dièz et al., 2005; Zucchi, 2005).

It seems that hierarchies are established within each warren and exchange of individuals among the warrens happens the non-breeding seasons; whereas, the rabbits use to leave in the already exhisting warrens (Mykytowycz and Gambale, 1965). The size of a group is modified by dispersing. Künkele and Von Host (1996), when studied the natal dispersal of European rabbits in an outdour enclosure for the period 1988-1990, noticed that 88% (first year) and 65% (second year) of juveniles dispersed within their first five months of life moving into other pre-existing territories (68%), settling unoccupied territories (21%), or becoming “non-territorial satellites” (11%). Males dispersed in a higher percentage than females (93 vs. 64%); whereas, it seemed that dispersing was not motivated by aggressions among juveniles or adults-juveniles.

The active life in the colony starts with the sunset: the rabbits come up to the surface in groups of three or more individuals and start feeding staying close to the burrows. In this way, they are able to detect the presence of any predator and escape more effectively (Roberts, 1988; Villafuerte and Moreno, 1997). Feeding behaviour, usually, takes 2 to 6 hours depending on the feed disposition, their age, their physiological state and the climate. Herbaceous plants are preferred, but also trunks and bark of young shrubs are consumed and in case of grazing absence, they dig the ground in search of seeds and roots.

Compared to the domestic rabbits, the wild ones are characterized by a great sensorial capacity and a great resistance to climatic conditions, especially cold. Cold does not disturb animal feeding, whereas snow, rain or strong winds interrupt them. During summer period, animals often present weight losses, because of feed scarsity.

Rabbits are very resistant to water deprivation and sometimes they manage to live only with the water derived by their alimentation. In nature, rabbits used to consume water during two very precise periods of day: night, when they exit from the burrows and dawn before they enter back to them.

One behaviour very important and on which rabbits devote a lot of their time is grooming. This may be self-grooming or allo-grooming, thus with the help of another member of the group. Mostly, it takes place the first morning hours (Cowan, 1987; McNitt et al., 1996; Zucchi, 2005).

In the study of Selzer and Hoy (2003) a lot of similarities have been noticed in a comparative study between wild and domestic groups of rabbits. More specifically, both wild and domestic rabbits devoted almost the same time in activities within the nest box (34.4 and 35.6%, respectively). Additionally, both of the groups preferred to spend more time together, in the same nest box, than using their own nest box that was available. Lastly, domestic rabbits were less shy compared to the wild ones, considering that they were spending more time outside the nest box during day and night.

Feeding/drinking behaviour

Rabbits being herbivores are equipped with adapted teeth, enlarged hindgut for fermentation and separation of the caecal content allowing the promotion of caecotrophy (McNitt et al., 1996). When fresh grass is available, they collect it piece by piece (Xu, 1996) choosing leaves rather than stems, young plants than old and green parts than dry, consuming, in this way, a diet rich in protein and digestible energy and less in fibre (McNitt et al., 1996). If grazing is allowed, they express their inclination for graminaceous plants (*Festuca sp.*, *Brachypodium sp.* or *Digitaria sp.*) and maybe for few dicotyledons (leguminous plants and compositae), while they are not very keen on carrots (Gidenne et al., 2010). During the consumption of grass, lower jaw's movement is very rapid reaching 170-200 movements/min. Sometimes, they use their forepaws to push aside their feed or throw it out of the feeder. Rabbit preference follows the order: pellet feeds, sweet feeds and fresh, juicy, green feeds, while porridge feeds are disliked (Xu, 1996). According to Gidenne et al. (2010), when the shift light/dark is 12L/12D, rabbits consume the 60% of solid feed (soft faeces are not included) during the dark period and by taking several meals during 24 hours.

Another very important behaviour is caecotrophy, while it is vital for the nutrition of rabbits, supplying their organism with proteins and vitamins B (bacterial derivations). This behaviour starts at about 25 days of age, coinciding with the beginning of the dry feed intake (Gidenne et al., 2010). In details, rabbits eliminate two types of faeces: the hard and the soft ones. The division of feed particles takes place in the hindgut and consequently the formulation of these two types of faeces occurs (McNitt et al., 1996). The soft faeces (caecotropes) are consumed directly from the anus. In order to recover them, rabbits turn around themselves, they bring their head between thighs, with pelvis forward (Samoggia, 1985) and they get the soft faeces upon being expelled from the anus, swallowing them without chewing. Sometimes, rabbits may collect the soft faeces also from the cage floor (Gidenne et al., 2010). It has been noticed that the time of most intense feeding takes place at about the same time that hard faeces excretion does it so, while the time of lowest feeding happens at about the same time with caecotrophy, which is mostly during morning (04.00-12.00 h). Attention should be given in case that rabbits may reject caecotrophy, which it may happen because of digestive disorders (Marai and Rashwan, 2003).

The drinking behaviour usually takes place during night (60%) and after feed intake (Marai and Rashwan, 2003). In order to drink, they suck water, then lift their head and swallow. After repeating these movements many times, often, they lick their lips to get the remaining water (Samoggia, 1985).

Urination/defecation behaviours

During the defecation of the hard faeces, thighs have a horizontal direction and no particular position is observed (Xu, 1996). Rabbits use to eliminate their droppings in a specific area inside the cage. This is of a great importance especially during lactation period (nest box should not be put close to this area) (McNitt et al., 1996).

The urination is realized by a vertical elimination downward and there is no difference between the two sexes. Other than an eliminative behaviour, urination may be, also, indicative of a territorial or aggressive behaviour. Male rabbits use to ‘spray’ their rivals with their urine making sure, in this way, their status in the hierarchy (McNitt et al., 1996). Sometimes, females mark their offsprings urinating onto them (Samoggia, 1985).

Social behaviour

Rabbits perceive their cage as their territory and they establish its boundaries by urination or ‘chinning’. Regarding ‘chinning’, rabbits excrete a colourless substance by the sebaceous and sudoriferous glands under the chin (submandibular glands) and mark objects by rubbing the chin on them (Marai and Rashwan, 2003; McNitt et al., 1996). There are also other glands that contribute to the olfactory communication; hence, the anal (rectum) and the inguinal glands (urogenital-anal line). The secretion of the anal glands also serves to mark individuals or territory. When a rabbit is placed in a new cage, it starts exploring it, bearing in mind its smell and, then, it marks it with its own odour (chinning, urination, defecation) (Marai and Rashwan, 2003; Xu, 1996).

However that pheromones (odours) are the principal mean of communication, rabbits communicate also by sight and hearing, whereas vocalisation is a less important mean. Sometimes they scream if they are terrified or they are in pain. Other types of vocalisation come from the offspring during lactation or from a female in case of a male approach, when she is not perceptive for mating. Additionally, male screaming after the completion of copulation is very characteristic of rabbit behaviour.

Going back to the communication by sight and hearing, rabbits realise a thumping with their hind feat, alerting the rest of the group for a possible danger. Another way to demonstrate alert is by exposing the internal side of the tail, turning it upwards. Very interesting is also the way that a dominant and a non-dominant animal communicate each other, with the latter makes clear its subjection by keeping its head, ears and tail bated until the dominant moves away (McNitt et al., 1996; Xu, 1996).

Furthermore, rabbits being social animals spend most of their time resting one close to the other or realizing allo-grooming to their group-mates. The recognition between them is realised through sniffing, while the group mates are characterised by the same odour (Xu, 1996).

Sexual behaviour

Usually, in order mating be fulfilled, the doe is brought into the buck cage. This method is followed so as fight not to break through, because of territorial reasons. In details, if the buck is placed into a does cage, the latter may attack to him to protect her area or the buck may not demonstrate any interest for the female, while he may be curious only for the new surrounding in which is put (McNitt et al., 1996). Does do not have a regular oestrous cycle, like other farm animals, but ovulation happens only after mating (10-13h). Of course, if does are not in estrus, mating cannot occur. Females are considered to be in estrus when they are receptive to the males. Moreover, a red colour of vulva may be also an indicator of estrus, but it is not, necessarily, a proof (Marai and Rashwan, 2003). Generally, the procedure starts with the buck approaching the doe, smelling her flunk, then the rump and the vulva. If the female is in full heat, she squats down; she raises her hindquarters and allows the buck to mount. The latter embraces tightly the flanks of the doe by his forelimbs and performs few quick copulatory movements until ejaculation, which is completed after the bucks falling sideways and performing a characteristic scream. Usually, after mating both male and female perform self-grooming. Contrary, if the doe is not in full heat, the buck may attempt several “courtship rituals”, like licking or poke his head under her abdomen and thus massage her udder etc. In case female is not receptive she will try to escape demonstrating aggressive behaviour against him (McNitt et al., 1996 ; Xu, 1996).

Stereotypic behaviour

The presence of abnormal behaviour in animals that live in captivity is very frequent. This behaviour is called *stereotypy*, which is "a repetitive, invariant behaviour with no obvious goal of function" (Dawkins, 2003). However, defining stereotypies meets difficulties and the classification of stereotypic behaviours is not yet clear. Nevertheless, the basic idea is that the behaviour of animals depends on the existence or not of a negative feedback. Thus, when an animal is highly motivated to do something and it does it so, then its motivation is reduced (negative feedback loop). For example, if an animal has the need to mate but this is impossible, because maybe it is not feasible to search for a mate, then the negative feedback fails to be accomplished and the animal stays highly motivated, which may result in "frustration-related stress" and, consequently, in stereotypic behaviours (Mason and Rushen, 2006).

Rabbits being curious use to interact a lot with the cage/pen elements (i.e. bite/lick the cage) or pull the hair by their co-specifics. In the case of cage/pen interaction, it is difficult to define if it is about a stereotypic behaviour or it is just an explorative behaviour.

Preference evaluation

Preference test is a simple way to investigate animals choice, while it "determines an animal's relative choice for different resources" (King, 2003). During this test the animals are given the opportunity to choose among different aspects of its surroundings. Preference test demonstrates "whether animals have a requirement for particular resources in their captive environment, sometimes with counterintuitive results" (King, 2003). For instance, rabbits can choose between plastic or wire floor types or between cage with or without mirrors, etc (Dalle Zotte et al., 2009a; Princz et al., 2008a).

This test has the advantages that it is practical, easy to apply and it gives a direct overview of what animals like. On the other hand, this test may be influenced by many factors, such as animals previous experience, stimuli that may distract animals or alter their choice (co-specifics, food resources), animals stress level, how they are given the choices, etc (Dawkins, 2003; Duncan, 2005; King, 2003).

At last, apart from these tests that animals make "a positive choice" to obtain their goal, the opposite tests are also used, hence tests that evaluate animals "tendency to avoid" (e.g. tests for avoiding painful or stressful situations) (Duncan, 2005).

Reactivity tests

"Emotional states are used as post-hoc variables to try to understand the animal's reaction to the test" (Dèsirè et al., 2002). Very often animal fear, as an emotional state, is examined through a variety of tests to give information about how animals interact with sudden or new stimuli and, usually, to further study the effect of the managerial system or housing system used by man. In these tests, animal reaction to negative events is called "emotionality", "reactivity" or "temperament" (Dèsirè et al., 2002). Few of the reactivity tests used for other species may be applied also on rabbits.

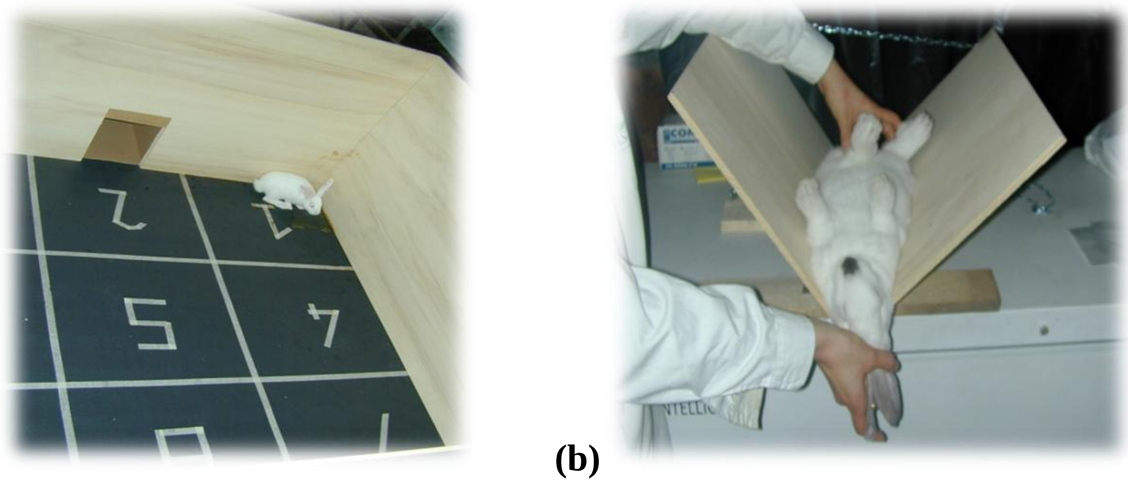
Open field or novel-arena test

The open field (OF) test is one of the fear tests that, firstly, it was applied for laboratory animals and then it has been further applied on rodents and now it constitutes one of the most popular test to evaluate emotions in farm species (Forkman et al., 2007; Meijsser et al., 1989; Walhs and Cummins, 1976).

In details, during this test a single animal is put in a novel open area from which escape is hampered by surrounding walls and its activity (movement, hopping, alerting, etc.) and defecation as a sign of timidity are then analyzed in order to make assumptions for their fear level (Figure 2a). The novel arena used for the test may be square, round or of some other shape. Floor and walls may be made of a variety of materials, such as wood, metal, glass etc., while it is very frequent to put an object inside the structure, like mirrors, food pellets, illuminations to evaluate animals interaction with a novel object.

Different factors may affect animals fear during the test and validity of the test itself. Of a great importance is the size of the arena, in relation with the species on which is used. For instance, for species that tend to hide, such as rabbits, the test could probably mean risk for predation, which is the real goal of the technique. As follows, this test is not recommended for species that are evolved to inhabit in fields of wide size (Forkman et al., 2007; Meijsser et al., 1989; Walhs and Cummins, 1976). Additionally, according to Forkman et al, (2007) species that stay together in groups may spend plenty of time in locomotion during the open field test, but this happens more for satisfying their need for group reinstatement reacting to social isolation, rather than curiosity. The same may happen also for collectively reared rabbits.

The open field test is still one of the most usable instruments to evaluate animals psychology. Its popularity leans on the fact that it is a simple and easy-to-apply method, it allows rapid evaluation of concrete behaviours and it is "sufficiently reliable under standardized conditions to give repeatable measures on an enormous range of independent variables" (Walhs and Cummins, 1976).



(a) **(b)**

Figure 2. Rabbits underwent the open field (a) and tonic immobility (b) tests.

Tonic immobility test

Tonic immobility (TI) or else hypnosis, is “a transitory immobility that can be induced experimentally in many animal species by short-lasting physical stimuli” (Aloisi et al., 1995). Usually, in rabbits is performed by putting animal, for a short period, in the supine position.

The tonic immobility test is based on the idea that the operator plays the role of a predator bringing out an anti-predator response that usually is the ‘death feigning’. The materials used for this test vary, but today most experiments for chickens use a cradle or cloth on a table top to induce immobility to the animals. Usually, the animal stays restrained with one hand put on the sternum and the other on its head and then it is left in this position for few seconds. Usually, it is evaluated the time spent to induce immobility to the animal and the time until the first movement of the head. On the other hand, in order to implement the test in rabbits (Figure 2b), the operator takes the animal and turns it on its back and onto his arm; he puts it on a U or V-shaped structure and leaves it there for few seconds (Aloisi et al., 1995; Forkman et al., 2007).

According to Forkman et al. (2007) the duration of immobility reflects the level of fear, while Erhard and Mendl (1999) stated that immobility represents a ‘passive response’ to a threatening situation. The fear level is affected by prior aversive treatment (increased duration of TI) or prior regular handling of animals by human (decreased duration of TI). Lastly, it is believed that the duration of TI is a more sensitive measure than the number of inductions needed (Forkman et al., 2007).

Novel object test

The novel object test is “an intuitively appealing test” which examines animals reaction to an unknown stimulus, which is believed to elicit animals fear (Forkman et al., 2007). The stimuli used are usually objects put by an operator on the floor of the cage or left to drop from the ceiling and stay on it or rotating from the ceiling (Figure 3a).

(a) **(b)**

Figure 3. Rabbits underwent the novel object (a) and human contact (b) tests.

The novel object test is a method that is widely used on many species and it is easy to apply and to repeat. On the other hand, one weakness of this test is that a “non-curious” animal and a “fearful” animal will both demonstrate a high latency to interact with the object, provoking confusion in the interpretation of the results (Forkman et al., 2007).

Other fear-based tests

According to Forkman et al. (2007), the exposure to human finds also appliance to farm animals. During this test an animal is either approached by a human (“Forced Approach test”) or is left to approach by its self a human (“Voluntary Approach test” or “human-contact test”) (Figure 3b).

Lastly, fear may additionally analyzed from the responses of animals facing one of their natural predators (“Predator test”) or to an impulsive sound or visual stimulation (“Startle test”) or to a signal associated with a negative event, e.g. an electrick shock (“Conditioned Fear test”).

Corticosterone concentration as stress indicator

As it has been already explained, the adrenal cortex excretes glucocorticoids contributing to daily functions (e.g. body energy balance, diurnal rhythms, copulation etc.), but when animals are facing a stressor glucocorticoids secretion in blood is rising. Consequently, glucocorticoids levels in blood have been used as indicator of stress (Sheriff et al., 2009).

However, a question that should be asked is: when is the best moment to collect the sample?. Hence, some researchers use glucocorticoids concentrations after the stressor, while others use the difference between post-stressor and baseline concentrations or the quota of the increase from the baseline sample (Buijs et al., 2011a). Furthermore, another factor is that blood sampling requires animal handling or confinement, i.e. procedures that *per se* are stressful and may influence the results obtained. Although, these effects are believed to be languished and non-invasive methods have been developed to analyze glucocorticoids metabolites (GCM) in substrates beyond blood.

In details, many researchers have developed such methods to determine metabolites in urine, saliva, milk or faeces. By these, analysis of glucocorticoids metabolites in faeces seems to be the best choice gaining great importance (Möstl and Palme, 2002). Measuring faecal GCM was first developed the years among 1970 and 1980 and the last two decades found application in a variety of species (Palme, 2005). This method is preferred because the sampling takes place without disturbing animals and because GCM in faeces represent the accumulation of glucocorticoids over several hours (Buijs et al., 2011a).

Of course, few points should be taken under consideration. According to Palme et al. (2005), faecal GCM concentration differs among species and between sexes, regarding the route of excretion. Differences have been, also, noted concerning the types of metabolites formed and excreted. In details, glucocorticoids are extensively metabolized in the liver and the metabolites are excreted as conjugates (sulfates or glucuronides) through the urine and the bile. In sheep and pigs, for instance, the majority of GCM are ether-extractable, whilst in chicken conjugated or polar unconjugated metabolites are more frequent (Palme et al., 2005). Lastly, there are also other critical points of concern: the time of sampling and the storage of the samples, the delay time of faecal excretion, the extraction procedures etc. (Palme, 2005).

To our knowledge, little information is available about the application of these measurements on rabbits (Buijs et al., 2011a; Comin et al., 2012; Monclús et al., 2006; Prola et al., 2013).

Objectives of the thesis

Animal welfare is a burning issue nowadays, especially in the European Countries and producers accept more and more pressure by society for “welfare-friendly” products. In fact, research within Europe has shown that from 25.000 consumers who participated, the 82% support animal welfare and vote for less intensive rearing methods (Eurobarometer, 2007), but on the other hand knowledge about how to achieve this goal is limited.

Concerning the sector of rabbits kept for meat production, a tendency to abandon the old practice of pair housing in cages (used in Italy and Hungary) and to move to group rearing is a fact and some countries (Germany, Netherlands) have already started working intensively on it by proposing restrictive rules for rabbit housing. Until now, studies have been conducted to compare welfare and productivity of rabbits kept in cages in small groups (<10 animals), whereas technical information on pen housing of rabbits in rather large groups are still missing.

At this point, it should be stressed also the lack of any European legislation concerning housing systems and rearing practices on which producers could be based in the countries which produce the majority of rabbit meat (i.e. Mediterranean Countries). Only the European Food and Safety Authority (EFSA) has put some light on the issue. EFSA in its document gave recommendations about the optimum cage dimensions, group size, stocking density, floor type of cages, environmental enrichment, etc (EFSA, 2005). In details, EFSA (2005) proposed rearing of fattening rabbits in small groups (7-8 animals), at density no more than 16 animals/m^2, with at least 625 cm^2/animal individual surface available and with live weight at slaughter that doesn’t exceed the 40 kg/m^2. The reference data above have been given basing on the results of research obtained in cage systems.

Having the above mentioned as a guideline, the present PhD Thesis was conformed with the goal to further indulge how alternative housing systems for fattening rabbits could be applied in a way to promote rabbits welfare and defend producers profit achieving the best productive results with less cost possible. The research was divided into four studies; the first focused on the comparison between bicellular cages and collective pens, as a first step, and, subsequently, within collective systems to find how productivity and welfare could be affected by different technical choices, with special emphasis on group size, slaughter age, group gender composition, stocking density, floor and environmental enrichment.

In details:

Pair vs. collective rearing

Trial No.1 – Welfare and productivity of growing rabbits housed in bicellular cages and collective pens: differences among rabbits reared in conventional bicellular cages vs. collective pens (>20 rabbits per pen) of different size (small *vs.* large), at different stocking densities (12 vs. 16 rabbits/m^2) and with wooden floor.

Within collective systems

Trial No. 2 – Effect of floor type, stocking density, slaughter age, and gender on welfare and productivity of growing rabbits housed in collective pens: rabbits reared in groups (20-27 animals) within pens with wooden *vs*. plastic slatted floor, at different stocking densities (12 vs. 16 rabbits/m^2) and slaughtered at two ages (76 vs. 83 days).

Trial No. 3 – Effect of environmental enrichment and slaughter age on welfare and productivity of growing rabbits housed in collective pens: rabbits reared in large groups (>27 animals) within pens with plastic slatted floor equipped with enrichment elements (plastic elevated platform and/or plastic tube) and slaughtered at two ages (68 vs. 75 days).

Trial No. 4 – Effect of different gender composition and floor combinations on welfare and productivity of growing rabbits housed in collective pens: rabbits reared in groups (16 animals) in communicating collective cages with various types of floor creating combinations (steel/plastic slat vs. steel/wire-net vs. wire-net/plastic slat), in groups of single or mixed-gender composition (females, males, females and males together).

Experimental part

Trial No. 1:

Welfare and productivity of growing rabbits housed in bicellular cages and collective pens

The results of the Trial No.1 have been published in:

Xiccato G., Trocino A., Filiou E., Majolini D., Tazzoli M., Zuffellato A., 2013. Bicellular cage vs. collective pen housing for rabbits: Growth performance, carcass and meat quality. Livestock Science 155: 407-414.

Trocino A., Filiou E., Tazzoli M., Bertotto D., Negrato E., Xiccato G., 2014. Behaviour and welfare of growing rabbits housed in cages and pens. Livestock Science 167: 305-314.

Introduction

The domestic rabbit (*Oryctolagus cuniculus L.*) is a nocturnal animal that, in the wild, lives in groups and spends most of its time underground. Under commercial intensive systems for meat production and according to the European Food and Safety Authority (EFSA, 2005), the welfare requirements of rabbits are not fulfilled because of constrains in social behaviours and movement possibilities. In fact, rabbits are usually kept in bicellular (two rabbits per cage) or small collective cages (4-6 rabbits per cage) at stocking densities higher than recommended (16 rabbits/m^2, at least 625 cm^2 per animal and a maximum of 40 kg slaughter live weight/m^2) (EFSA, 2005; Trocino and Xiccato, 2006). Nevertheless, rearing rabbits in collective cages or in pens with large groups (>10 animals) can impair carcass and meat quality (Combes and Lebas, 2003; Szendrő and Dalle Zotte, 2011), not to mention the negative consequences linked to the increased aggressiveness among cage mates (Szendrő et al., 2009b). In groups of two or more animals, both the cage/pen dimensions and the group size may affect the expression of different behaviours and the quality of social interactions (Buijs et al., 2011a, 2011b, 2011c; Morisse and Maurice, 1997; Postollec et al., 2006, 2008). Unsuitable environment and social relationships/isolation may negatively affect stress level and behaviours and, consequently, animals immune response and growth performance (Forkman et al., 2007; Koolhaas et al., 1999; Sevi, 2009).

Useful information regarding rabbit welfare may be derived from behavioural indicators based on ethograms and reactivity to humans or a new environment and from physiological indicators such as the levels of glucocorticoids or their metabolites. The tonic immobility test may be used to assess the level of fear that rabbits have towards humans (Ferrante et al., 1992; Verwer et al., 2009) and the open field test may be used to measure fear in an unknown environment (Ferrante et al., 1992; Meijsser at al., 1989; Trocino et al., 2013). Glucocorticoids or their metabolites may be measured in blood and in other matrices, such as faeces and hair (Monclús et al., 2006; Palme, 2012), even though these measurements have only been used recently in rabbits kept under commercial conditions (Buijs et al., 2011a; Comin et al., 2012; Prola et al., 2013).

The present study aimed at evaluating whether and how growth performance, behaviour, fear and stress level of rabbits may be affected by the housing system (wire net bicellular cages with top vs. collective open-top pens with wooden slatted floor) and, within collective pens, by pen size (small vs. large) and stocking density (12 vs. 16 rabbits/m^2).

Materials and Methods

Animals and housing

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 the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

The trial was performed in a non-air-conditioned stable during the months of September and October. The temperature ranged from 12 to 25°C, and a natural photoperiod (11-13 h daylight) was maintained.

A total of 456 rabbits of both genders from the Hyplus crossbred line (Hypharm, Groupe Grimaud, Roussay, France) were given *ad libitum* access to a commercial diet for growing rabbits. Rabbits were individually identified by ear marking and their performance was controlled from weaning(35 d of age) to 76 d. Eighty rabbits were put into 40 bicellular cages (28 × 40× 28 cm-height; available surface per rabbit: 560 cm^2). The floors, roofs and walls of cages were made of wire net (Figure 1.1). The cages were equipped with a nipple drinker and a feeder for the manual distribution of feed. The remaining 376 rabbits were put into 12 collective pens located in the same room with the bicellular cages (Figure 1.1). Eight small pens (1.40 × 1.20 m = 1.68 m^2) with 20-27 animals/pen and 4 large pens (1.40 × 2.40 m = 3.36 m^2; 40-54 animals/pen) with 40-54 animals/pen were used (Figure 1.2). Within each type of pen, 12 and 16 animals/m^2 stocking densities were tested.

(a) **(b)**

Figure 1.1. Bicellular wire-net cages (a) and open-top collective pens (b) equipped with wooden slat floor.

The collective pens had wooden slat floors (sections of the slat: 8 × 8 cm; distance between the slats: 3 cm), wooden side walls (105 cm-height), and front and back walls covered by a galvanised wire net. The pens were equipped with 4 (small pens) or 8 (large pens) feeders (43.5 × 11.5 × 30.0 cm-height) for the manual distribution of feed at the side walls and 4 (small pens) or 8 (large pens) nipple drinkers on the back side.

(a) **(b)**

Figure 1.2. Small (a) and Large (b) collective pens, respectively.

The line of bicellular cages was in front of the line of pens and the two lines were separated by a 2.0-m-wide alley used by operators for controls. On the back of the pens, a 3-m-wide alley was available for the passage of people entering the stable.

The rabbits were allocated into five experimental groups that differed in the housing system, the pen size and the stocking density, as detailed below:

- group B18: 40 bicellular cages with top (2 rabbits/cage, 18 animals/m^2, n=80 rabbits);
- group S12: 4 open-top small collective pens (20 animals/pen, 12 animals/m^2, n=80 rabbits);
- group S16: 4 open-top small collective pens (27 animals/pen, 16 animals/m^2, n=108 rabbits);
- group L12: 2 open-top large collective pens (40 animals/pen, 12 animals/m^2, n=80 rabbits);
- group L16: 2 open-top large collective pens (54 animals/pen, 16 animals/m^2, n=108 rabbits).

Growth performance and sanitary status

Individual live weight and cage feed intake were recorded once per week. The health status of rabbits was monitored daily to detect any clinical sign of disease.

Behavioural evaluation during 24-h

The behaviour of the rabbits was video-recorded in 20 bicellular cages (40 rabbits), one L12 pen (40 rabbits), one L16 pen (54 rabbits), two S12 pens (40 rabbits) and two S16 pens (54 rabbits) allowing observation of 228 rabbits. The video-recording was performed for 24 hours at 52 and 73 d. Two minutes were analysed for each half-hour from each cage and pen. During the night, minimal light (about 20 lux) was used to avoid disturbing the activities of the rabbits. The following behaviours were analysed as percentage of the observed time: resting (crouched body, with abdomen in contact with the floor, or stretched body, with both fore and hind legs stretched beside the abdomen in contact with the floor), self-grooming (licking, nibbling, scratching the own head or body or stroking the forepaws over it), allo-grooming (licking or nibbling a pen/cage-mate), feeding (head in feeder), drinking (mouth in contact with drinking nipples), moving (displacing the whole body), running (displacing the whole body rapidly), standing still (sitting, looking around without doing anything), biting/licking (biting or licking elements of the pen) and sniffing (standing or moving with forelegs while sniffing around) (Dal Bosco et al., 2002; Morisse et al., 1999; Trocino et al., 2013). The occurrence (n) of rearing/alert (up-heaving on hind legs), hops (displacing the body by a hop), aggressive interactions (threatening, biting, attacking, fighting, pushing, chasing or scrathing a pen/cage-mate) and stereotypic behaviours (intense and repeated biting of cage wire net) was recorded.

Reactivity towards man (Tonic immobility test)

The test of tonic immobility was performed at 55 and 72 d on a total of 160 rabbits (80 rabbits × 2 ages). The test was performed on different animals for the two ages to avoid the rabbits becoming accustomed to the test. One rabbit per housing system was tested in sequence for 16 replications at each age. In total, 32 rabbits from each group (B18 group: 1 animal × 16 bicellular cages × 2 ages; L12 group: 8 animals × 2 pens × 2 ages; L16 group: 8 animals × 2 pens × 2 ages; S12 group: 4 animals × 4 pens × 2 ages) were tested.

The test was performed in a contiguous room in the same barn where the cages and pens were placed. The operator took the rabbit out of the cage and induced to it immobility by turning the animal on its back and onto his arm. The immobile rabbit was laid down on its back on a V-shaped wooden structure (Ferrante et al., 1992). A maximum of three attempts to induce immobility were performed, and rabbits were left in the immobility condition for no more than

180 s. The number of attempts necessary to induce immobility and the total duration of immobility were recorded per rabbit.

Reactivity towards an unknown environment (Open field test)

The test of open field was performed at 56 and 75 d on a total of 160 rabbits (80 rabbits × 2 ages). The rabbits tested had not been previously submitted to the tonic immobility test; whereas different animals used for the two ages. One rabbit from each housing system was tested in sequence for 16 replications as described for the tonic immobility test.

The test was performed contemporary in two arenas (1.5 × 1.5 m) with 0.80-m-high wooden walls and plastic floors divided into nine numbered squares. The arenas were located in a contiguous room in the same barn where the cages were placed. The total duration of each test was 10 minutes per animal. Each rabbit was put in a closed wooden box (22 × 30 × 30 cm-high) connected to the arena by a sliding door. After one minute, the sliding door was opened. The number of attempts rabbit made and the time (latency) spent to enter the arena were recorded for one 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 behaviour of the rabbit was video-recorded for eight minutes.

The following behaviours were considered by Ferrante et al. (1992), Meijsser et al. (1989), and Trocino et al. (2013): total displacements, the number of squares that each rabbit crossed in the arena; central displacements, the number of times the rabbit crossed the square in the centre of the arena; movement, time spent in moving with fore and hind legs among squares; running, time spent in running among squares; exploration, the time spent moving with forelegs or standing while sniffing and looking around inside the same square; escape attempts, the number of rapid runs towards the corners of the arena; hops, the number of times the rabbit completely displaced its body by a hop; standing still, the time the rabbit spent still with its fore and hind legs unstretched and on the ground; rearing, the number of times the rabbit reared up on its hind legs; grooming, the time spent self-grooming; digging, the time spent digging inside the arena; biting, the time spent biting elements of the pen; resting, the time spent inactive with the body touching the floor and fore and/or hind legs stretched on the floor; defecation, the number of times the rabbit defecated; and urination, the number of times the rabbit urinated.

Corticosterone determination in hard faeces and hair

At 63 and 70 d of age, samples of hard faeces and hair were collected from a total of 120 rabbits (12 rabbits × 5 experimental groups × two ages), i.e. 24 rabbits were tested from each group (B18 group: 1 animal × 12 bicellular cages × 2 ages; L12 group: 6 animals × 2 pens × 2 ages; L16 group: 6 animals × 2 pens × 2 ages; S12 group: 3 animals × 4 pens × 2 ages; S16 group: 3 animals × 4 pens × 2 ages).

A number of pellets of hard faeces were collected between 16.00 and 18.00 h directly from each animal by applying a gentle pressure at the perianal area. The hair sample was collected by gently pulling hair from the back and hind legs.

The corticosterone levels in hard faeces and hair were measured by microtitre radioimmunoassay (RIA) using species-specific antibodies (Biogenesis, Poole, England, UK) as described in detail by Simontacchi et al. (2009). Before RIA, steroids were extracted with 8 mL of diethyl ether from hard faeces (50 mg) and hair (30 mg) that were thawed and pulverised in liquid nitrogen. Dry extracts were then dissolved in 1 mL of phosphate buffer (PBS, pH 7.2), and various aliquots were used for the assay depending on the steroid content in the sample. The anti-corticosterone serum showed the following cross-reactions: corticosterone 100%, 11-deoxycorticosterone 1%, cortisol 0.1%, progesterone 0.02%, testosterone <0.01%. To validate RIA corticosterone measurements in faeces and hair, parallelism and intra- and interassay tests were performed. The tests of parallelism were carried out by analysing serially diluted extracts from the matrices with high corticosterone concentrations. The sensitivity of the assay was 1.5 pg/well and was defined as the dose of the hormone at 90% binding.

The corticosterone assay exhibited excellent precision and reproducibility (hair: intra-assay coefficient of variation 1.61%, inter-assay coefficient of variation 12.5%; hard faeces: intra-assay coefficient of variation 2.99%, inter-assay coefficient of variation 13.5%). The mean recovery rates of corticosterone added to hair and faeces were 82% and 66%, respectively.

Commercial slaughter

At 77 d, 396 rabbits were individually processed in a commercial slaughterhouse. Of these, 78 rabbits came from 39 bicellular cages (one cage was excluded because of mortality); 79 rabbits were taken from the four S12 pens (all animals present in these pens); 80 rabbits (20 per pen) were selected out of the four S16 pens (containing 27 rabbits each); 79 rabbits were taken from the two L12 pens (all animals present in these pens); and 80 rabbits (40 per pen) were selected out of the two L16 pens (containing 54 rabbits each). Rabbits selected (pens S16 and L16) were representative of their experimental group in terms of live weight and variability. The slaughtering took place after 6 hours of fasting and a transport of approximately 1 h. The animals were stunned by electro-anaesthesia, followed by jugular exsanguination. After 2.5 h of cooling at 3-4°C, the commercial carcasses were weighed to measure the individual dressing out percentages as (cold carcase weight ÷ liveweight) × 100.

Statistical analysis

Cage data for growth performance were analysed by ANOVA with the housing system as the main effect, using the PROC GLM of SAS (SAS, 2013). Individual data for slaughter results were analysed by using the PROC MIXED of SAS with the housing system as the main effect and the cage/pen as the random effect. A Bonferroni t test was used to compare the means by group of the housing systems. Differences between the means of the type of housing system (bicellular cage vs. collective pens), dimensions of collective pens (small vs. large), and stocking density within collective pens (12 vs. 16 rabbits/m^2) were tested by the contrast statement of SAS.

The whole data set of behavioural data collected at two ages on bicellular cages and collective pens was firstly analysed by a mixed model in SAS (SAS, 2013), i.e. PROC GLIMMIX with housing system (bicellular cages vs. collective pens), age and their interaction as fixed effects and time of day as a random effect. An underlying Poisson distribution was assumed for data expressed in percentage of the time of observation; a binomial distribution was used for behaviours that seldom happened and occurred (i.e. biting, running, rearing, hops, aggressions, stereotypic behaviours). Data from the same cage/pen were treated as repeated measures.

The behavioural data set collected at two ages on collective pens was analysed with PROC GLIMMIX to test the effect of pen size (large vs. small), stocking density (12 vs. 16 rabbits/m^2) and age and their interactions with age as a fixed effect and time as a random effect. The interaction between pen size and stocking density was not tested because repeated units were not sufficient (only one cage L12 and one cage L16 were video-recorded). Data from the same pens were treated as repeated measures.

The data of the reactivity tests (tonic immobility test, open field test) from bicellular cages and collective pens were firstly analysed by PROC GLIMMIX with housing system (bicellular cages vs. collective pens), age and their interaction as fixed effects, and cage/pen as random effect. An underlying Poisson distribution was assumed for data expressed in percentage of the time of observation. Then, the data from the reactivity tests performed on rabbits of collective pens were analysed by PROC GLIMMIX to test the effect of pen size (large vs. small), stocking density (12 vs. 16 rabbits/m^2) and age and their interactions as fixed effects and cage/pen as a random effect.

The data of corticosterone levels in faeces and hair was analysed by ANOVA using the PROC MIXED with experimental group, age and their interactions as factors of variability and the pen/cage as random effect. The CONTRAST statements were used to test the effect of the type of housing (bicellular cages vs. collective pens) and, within collective systems, pen size (small vs. large) and stocking density (12 vs. 16 rabbits/m^2).

Differences among means with P<0.05 were accepted as representing statistically significant differences.

Results

Growth performance and sanitary status

The housing system significantly affected the growth performance of the rabbits during the trial (Table 1.1). During the first period (35 to 54 d of age), the rabbits in collective pens showed lower daily weight gain (17.4%), feed intake (14.1%) and feed efficiency (9.2%) compared with the rabbits in collective pens (probability of the contrast bicellular vs. collective, P<0.01) and showed lower live weight at 54 d (1865 vs. 1687 g; P<0.001). During fattening (from 54 to 76 d of age), the rabbits from different groups had a similar growth rate, but those kept in collective pens consumed less (6.9%) and showed a higher feed efficiency (6.9%) compared with those in bicellular cages. Consequently, throughout the trial, the rabbits in collective pens had lower daily weight gains and feed intakes, and lower final live weights compared with the rabbits in bicellular cages, without differences in feed efficiency.

Within the collective systems, growth performance was not affected by the pen dimensions or the stocking density.

During the trial, two animals from one bicellular cage died and two other rabbits (one from L12 and one from S12 pens) were excluded because their live weight at slaughter was below the commercial weight accepted (2.0 kg).

Behaviour during the 24-h period

The rabbits kept in collective pens spent less time in feeding (7.8 vs. 10.9% of observed time; P<0.01), allo-grooming (0.65 vs. 1.58%; P<0.001) and more time in displacing (moving: 0.81 vs. 0.35%; P<0.001) and resting (82.1 vs. 77.7% of observed time; P<0.01) compared to those housed in bicellular cages. The rabbits from the pens were observed running, rearing and hopping and did not exhibit stereotypic behaviours (i.e., intense and repeated biting of cage wire net) compared to the latter rabbits (Table 1.2). Some significant interactions were measured between the housing system and the age: feeding time decreased from 52 to 73 d of age only in rabbits kept in collective pens; conversely, allo-grooming increased and moving and sniffing decreased from 52 to 73 d of age only in rabbits housed in bicellular cages.

Table 1.1

Rabbits performance from 35 days (weaning) until 76 days of age: effect of housing system, pen size and stocking density

	Housing system					P-value				RSD
	Bicellular	Collective				Housing system	Bicellular vs. Collective	Small vs. Large	12 vs. 16 rabbits/m^2	
		Small size		Large size						
	18 rabbits/m^2	12 rabbits/m^2	16 rabbits/m^2	12 rabbits/m^2	16 rabbits/m^2					
Item	B18	S12	S16	L12	L16					
No. of cages	39	4	4	2	2					
Live weight (g)										
35 d	864	851	864	864	859	0.99	0.78	0.88	0.89	48
54 d	1865^{B}	1709^{A}	1693^{A}	1667^{A}	1681^{A}	0.001	<0.001	0.70	0.99	116
76 d	2839	2655	2702	2598	2665	0.08	<0.01	0.68	0.62	185
From 35 to 54 d										
Weight gain (g/d)	52.7^{B}	45.2^{A}	43.6^{A}	42.2^{A}	43.2^{A}	<0.001	<0.001	0.54	0.92	4.4
Feed intake (g/d)	126^{B}	111^{A}	108^{A}	107^{A}	107^{A}	<0.001	<0.001	0.67	0.80	10
Feed efficiency	0.417	0.405	0.403	0.394	0.403	0.13	0.01	0.57	0.72	0.0-17
From 54 to 76 d										
Weight gain (g/d)	44.3	43.0	45.9	42.3	44.8	0.93	0.87	0.79	0.44	5.5
Feed intake (g/d)	174	162	166	160	160	0.30	0.03	0.69	0.82	16
Feed efficiency	0.254	0.266	0.277	0.265	0.279	0.12	0.01	0.93	0.37	0.0-20
From 35 to 76 d										
Weight gain (g/d)	48.2^{B}	44.0^{A}	44.8^{A}	42.3^{A}	44.0^{A}	0.04	<0.01	0.60	0.59	3.9
Feed intake (g/d)	152^{B}	139^{A}	139^{A}	135^{A}	136^{A}	0.02	0.001	0.66	0.95	12
Feed efficiency	0.317	0.318	0.322	0.312	0.324	0.83	0.60	0.85	0.31	0.0-13
Dressing percentage (%)	60.5^{B}	59.4^{A}	60.0^{AB}	59.3^{A}	59.5^{A}	<0.001	<0.001	0.28	0.05	1.6

^{A,B}Within a row, housing system means without a common superscript differ ($P \leq 0.01$)

Time spent in resting with stretched body decreased from 52 to 73 d of age ($P<0.001$), but the reduction was more pronounced in rabbits in bicellular cages (from 12.7 to 0.87% of observed time) than in those in collective pens (from 9.32 to 3.61%).

Within the collective housing systems, rabbits in the larger pens shared a numerical decrease in the time rabbits spent in eating (8.30 vs. 6.55%), self-grooming (6.39 vs. 5.47%), sniffing (1.53 vs. 1.19%), and more time in resting (81.1 vs. 84.1%) (Table 1.3). During the resting time, the rabbits housed in the small pens stayed less with their body crouched compared to the rabbits in large pens (73.6 vs. 79.8% of observed time; $P<0.01$).

In collective systems, changes of stocking density *per se* had only a weak effect but showed some significant interactions with age ($P<0.01$) (Table 1.3): feeding time was less in rabbits stocked at 12 rabbits/m^2 compared to those at 16 rabbits/m^2 at 52 d of age (7.19 vs. 9.49%) whereas differences disappeared at 73 d of age (6.60 vs. 6.35%); time spent in grooming was similar at 52 d of age (self-grooming: 4.49 vs. 4.84%; allo-grooming: 0.45 vs. 0.21%) but differed at 73 d of age (self-grooming: 7.81 vs. 6.55%; allo-grooming: 0.35 vs. 1.11%). The increase in age from 52 to 73 d significantly affected the behaviour of the rabbits (Table 1.3). The older rabbits spent more time self-grooming, allo-grooming, sniffing, and running. Older rabbits also exhibited more hops and reduced feeding time. The resting time was slightly less at 73 d (81.7%) than at 52 d of age (83.6%) and the stretched position was less preferred (8.37 to 3.24%) by older rabbits.

Table 1.2

Behaviours during video-recording (% of budget time or number of events [a]): effect of bicellular cages vs. collective pens and age

Housing system (H)	Bicellular cages	Collective pens	Bicellular cages	Collective pens	P-value		
Rabbit age (A)	52 d	52 d	73 d	73 d	H	A	H x A
Observations (n)	480	144	480	144			
Drinking (%)	0.72	0.99	0.83	1.03	0.17	0.37	0.43
Feeding (%)	11.0	8.62	10.8	6.89	<0.01	<0.001	<0.001
Self-grooming (%)	5.55	4.91	8.30	7.30	0.24	<0.001	0.86
Allo-grooming (%)	1.13	0.58	2.02	0.71	<0.001	<0.001	0.02
Moving (%)	0.45	0.80	0.24	0.81	<0.001	<0.001	<0.001
Sniffing (%)	2.40	1.18	1.21	1.68	0.332	<0.01	<0.001
Total resting (%)	78.7	82.9	76.6	81.3	<0.01	<0.01	0.55
With stretched body (%)	12.7	9.32	0.87	3.61	<0.001	<0.001	<0.001
With crouched body (%)	66.0	73.5	75.7	77.7	<0.01	<0.001	<0.001
Biting (n)	0.00	0.05	0.00	0.03	n.e.	n.e.	n.e.
Running (n)	0.00	0.18	0.00	0.37	n.e.	n.e.	n.e.
Rearing (n)	0.00	0.04	0.00	0.06	n.e.	n.e.	n.e.
Hops (n)	0.00	0.03	0.00	0.12	n.e.	n.e.	n.e.
Aggressions (n)	0.00	0.00	0.00	0.02	n.e.	n.e.	n.e.
Stereotypies (n)	0.02	0.00	0.03	0.00	n.e.	n.e.	n.e.

n.e. non-estimable

[a]The sum of behaviours may be less than 100% because of some minor behaviours (biting, running, standing still) that took a minor percentage of budget time but were not suitable for the statistical analysis and are not included in table

Table 1.3

Behaviours during video-recording (% of budget time or number of events [a]): effect of pen size, stocking density and age in rabbits reared in collective pens

	Pen size (P)				Stocking density (rabbits/m^2) (S)				P-value[b]				
	Small	Large	Small	Large	12	16	12	16					
Rabbit age (A)	52 d	52 d	73 d	73 d	52 d	52 d	73 d	73 d	P	S	A	P x A	S x A
Observations (n)	96	48	96	48	72	72	72	72					
Drinking (%)	0.99	0.93	0.92	1.26	1.13	0.82	1.07	1.08	0.65	0.57	0.37	0.12	0.16
Feeding (%)	9.06	7.53	7.54	5.56	7.19	9.49	6.60	6.35	0.08	0.39	<0.001	0.21	<0.01
Self-grooming (%)	5.27	4.12	7.50	6.81	4.49	4.84	7.81	6.55	0.06	0.57	<0.001	0.17	<0.01
Allo-grooming (%)	0.79	0.12	0.34	1.13	0.45	0.21	0.35	1.11	0.30	0.53	<0.001	<0.001	<0.001
Moving (%)	0.85	0.68	0.87	0.67	0.73	0.79	0.85	0.69	0.11	0.68	0.96	0.90	0.27
Sniffing (%)	1.39	0.67	1.66	1.72	1.20	0.78	1.76	1.62	0.07	0.16	<0.001	<0.01	0.10
Total resting (%)	81.4	85.7	80.7	82.5	84.4	82.7	80.9	82.4	0.06	0.94	0.09	0.28	0.13
With stretched body (%)	10.4	6.34	4.36	2.11	10.3	6.44	3.03	3.04	<0.001	0.10	<0.001	0.08	<0.001
With crouched body (%)	70.7	79.2	76.4	80.3	73.6	76.1	77.7	79.0	<0.01	0.34	<0.01	0.03	0.56
Biting (n)	0.03	0.07	0.01	0.08	0.05	0.04	0.01	0.04	0.03	0.51	0.36	0.29	0.35
Running (n)	0.14	0.25	0.31	0.45	0.17	0.21	0.46	0.31	0.06	0.61	<0.01	0.74	0.24
Rearing (n)	0.05	0.02	0.04	0.10	0.04	0.02	0.07	0.06	0.93	0.51	0.28	0.17	0.84
Hops (n)	0.03	0.02	0.08	0.19	0.01	0.04	0.13	0.12	0.92	0.31	0.02	0.24	0.24

[a]The sum of behaviours may be less than 100% because of some minor behaviours (biting, running, standing still) that took a minor percentage of budget time but were not suitable for the statistical analysis and are not included in table

[b]Probability of P x S x A non-estimable

Reactivity towards man and environment

In the tonic immobility test, the percentage of sensitive rabbits (which turned immobile within three attempts) was significantly lower in the rabbits kept collectively compared to those reared in bicellular cages (76.6 vs. 93.8%; P=0.03) (Table 1.4) regardless of pen size and stocking density (Table 1.5). Among the rabbits sensitive to the test, neither the number of attempts nor the time of immobility differed according to the housing system (bicellular vs. collective) (Table 1.4) or the conditions of the collective systems (pen size, stocking density) (Table 1.5). The total duration of immobility significantly decreased (41. vs. 28.9 s; P<0.001) when the age of rabbits increased from 55 to 72 d (Table 1.4). Within collective systems, at 55 d of age rabbits exhibited a higher immobility when kept in large pens compared to those in small pens and when housed at 12 rabbits/m^2 compared to those at 16 rabbits/m^2. Differences among groups by pen size or by stocking density disappeared at 72 d of age (probability of the interaction pen size x age (P<0.001) and stocking density x age (P<0.05)) (Table 1.5).

In the open field test, the rabbits kept collectively in pens crossed fewer squares (30.8 vs. 47.5; P<0.001), moved less (40.4 vs. 57.2 s; P<0.001) and explored less (345 vs. 371 s; P<0.001) the arena than the rabbits from the bicellular cages and stood still for a longer period of time (82.1 vs. 38.7 s; P<0.001) (Table 1.6). Significant interactions between housing system and age were also measured: the number of total displacements was much lower in rabbits of collective pens than in those of bicellular cages at 75 d of age rather than at 56 d of age. A similar trend was recorded for the number of central displacements and the time spent running. Time spent biting measured at 56 d of age was less than at 75 d of age (Table 1.6). Within the collective systems, pen size had no effect whereas stocking density only affected the time that the rabbits groomed (P=0.02) (Table1.7). Significant interactions between pen size and age were measured for minor behaviours like running, grooming, biting and digging (Table 1.7). The major interaction between stocking density and age (P<0.001) was recorded for the latency to enter the arena which was higher in rabbits housed at 12 animals/m^2 compared to those at 16 animals/m^2 at 56 d of age and lower in the rabbits of the former group at 75 d of age (Table 1.7).

When the age of rabbits increased, the rabbits in collective pens showed a lower number of total and central displacements, spent less time moving, running and grooming, whereas they increased the time spent standing still and biting (Table 1.7).

Table 1.4

Response to the tonic immobility test: effect of housing system and age

Housing system (H)	Bicellular cages	Collective pens	Bicellular cages	Collective pens	P-value		
Rabbit age (A)	55 d	55 d	72 d	72 d	H	A	H x A
Rabbits (n)	16	64	16	64			
Sensitive rabbits (%)[a]	87.5	81.3	100	71.9	0.03	0.43	0.06
Attempts (n)	1.94	2.19	1.94	2.30	0.30	0.86	0.86
Immobility (s)	46.5	36.4	31.5	26.3	0.47	<0.001	0.31

[a]Rabbits that exhibited immobility within three attempts

Table 1.5

Response to the tonic immobility test: effect of pen size, stocking density and age in rabbits reared in collective pens

	Pen size (P)				Stocking density (rabbits/m^2) (S)				P-value[b]					
	Small	Large	Small	Large	12	16	12	16						
Rabbit age (A)	55 d	55 d	72 d	72 d	55 d	55 d	72 d	72 d	P	S	A	P x A	S x A	P x S
Rabbits (n)	32	32	32	32	32	32	32	32						
Sensitive rabbits (%)[a]	81.3	81.3	71.9	71.9	84.4	78.1	68.7	75.0	0.99	0.99	0.21	0.52	0.67	0.84
Attempts (n)	2.03	2.34	2.28	2.31	2.05	2.34	2.78	2.31	0.51	0.51	0.66	0.58	0.58	0.72
Immobility (s)	31.8	42.2	27.0	27.1	41.9	32.0	29.1	25.2	0.43	0.26	<0.001	<0.001	0.05	0.86

[a]Rabbits that exhibited immobility within three attempts

[b]Probability of P x S x A, P>0.05

Table 1.6

Behaviours during the open field test: effect of housing system and age

Housing system (H)	Bicellular cages	Collective pens	Bicellular cages	Collective pens	P-value		
Rabbit age (A)	56 d	56 d	75 d	75 d	H	A	H x A
Rabbits (n)	16	64	16	64			
Entered animals[a,b] (%)	75.0	51.6	62.5	54.7	0.11	0.99	0.37
Attempts (n)	0.31	0.34	0.31	0.36	0.74	0.94	0.95
Latency (s)	34.2	43.3	40.8	41.6	0.24	0.04	0.001
Total displacements (n)	46.2	34.1	48.8	27.5	<0.001	<0.01	<0.001
Central displacements (n)	0.38	0.51	1.00	0.23	0.28	0.76	<0.01
Exploration (s)	371	345	371	344	<0.001	0.98	0.88
Movement (s)	58.9	42.7	55.4	38.1	<0.001	<0.01	0.34
Running (s)	2.56	2.41	3.56	1.09	0.19	0.06	<0.001
Standing still (s)	38.2	77.5	39.1	86.7	<0.001	0.02	0.15
Grooming (s)	1.50	1.58	1.75	1.96	0.86	0.23	0.84
Biting (s)	3.56	2.41	6.31	6.56	0.68	<0.001	0.03
Digging (s)	1.88	0.49	1.00	0.45	0.12	0.07	0.19

[a]Rabbits that entered the arena spontaneously within 60 s

[b]Probability of χ^2 test

Table 1.7

Behaviours during the open field test: effect of pen size, stocking density and age in rabbits reared in collective pens

	Pen size (P)				Stocking density (rabbits/m^2) (S)				P-value[c]					
	Small	Large	Small	Large	12	16	12	16						
Rabbit age (A)	56 d	56 d	75 d	75 d	56 d	56 d	75 d	75 d	P	S	A	P x A	S x A	P x S
Rabbits (n)	32	32	32	32	32	32	32	32						
Entered animals[a,b] (%)	46.9	50.0	40.6	50.0	46.9	50.0	46.9	43.0	0.48	0.99	0.72	0.86	0.97	0.80
Attempts (n)	0.41	0.28	0.27	0.43	0.37	0.31	0.31	0.38	0.85	0.98	0.98	0.17	0.53	0.40
Latency (s)	43.7	43.3	39.9	43.7	45.7	41.4	39.9	43.6	0.68	0.96	0.13	0.07	<0.001	0.37
Total displacements (n)	32.7	36.1	27.6	27.9	35.7	33.2	28.8	26.8	0.66	0.55	<0.001	0.17	0.99	0.77
Central displacements (n)	0.09	0.52	0.00	0.00	0.39	0.13	0.00	0.00	0.98	0.98	<0.001	0.98	0.98	<0.001
Exploration (s)	352	334	348	338	343	342	340	345	0.10	0.83	0.93	0.27	0.38	0.25
Movement (s)	43.3	41.4	39.5	36.1	41.5	43.2	36.9	38.6	0.47	0.64	<0.001	0.44	0.92	0.71
Running (s)	2.12	3.27	0.33	1.79	2.63	2.63	0.58	1.02	0.14	0.69	<0.001	<0.01	0.20	0.28
Standing still (s)	69.6	82.8	78.3	96.1	74.8	77.1	89.9	83.7	0.17	0.88	<0.001	0.42	0.01	0.26
Grooming (s)	32.7	36.1	27.6	27.9	35.7	33.2	28.8	26.8	0.17	0.02	0.01	<0.001	0.07	0.81
Biting (s)	1.35	2.76	8.08	4.52	1.74	2.14	5.37	6.81	0.88	0.62	<0.001	<0.001	0.88	0.28
Digging (s)	0.18	0.22	0.00	0.00	0.29	0.13	0.35	0.00	0.28	0.99	0.99	<0.001	0.99	0.25

[a]Rabbits that entered the arena spontaneously within 60 s
[b]Probability of χ^2 test
[c]Probability of P x S x A, P>0.05

Corticosterone in hard faeces and hair

The corticosterone level in hard faeces did not vary with treatments (Figure 1.2a), whereas the hair corticosterone level was higher in the rabbits reared in collective pens compared to those in bicellular cages (15.7 vs. 6.7 ng/g; P<0.001) and in rabbits at 70 d of age compared to those at 63 d of age (17.0 vs. 10.7 ng/g; P<0.001) (Figure 1.2b). A significant interaction between the housing system and the age of animals was also measured; the hair corticosterone levels increased with age only in rabbits reared in collective systems (6.5 ng/g, 10.5 ng/g, 14.1 ng/g, 11.0 ng/g and 11.6 ng/g in rabbits of the groups B18, L12, L16, S12 and S16, respectively, at 63 d of age and 6.8 ng/g, 19.7 ng/g, 21.2 ng/g, 17.7 ng/g and 19.6 ng/g at 70 d of age in the same groups) (Figure 1.2b).

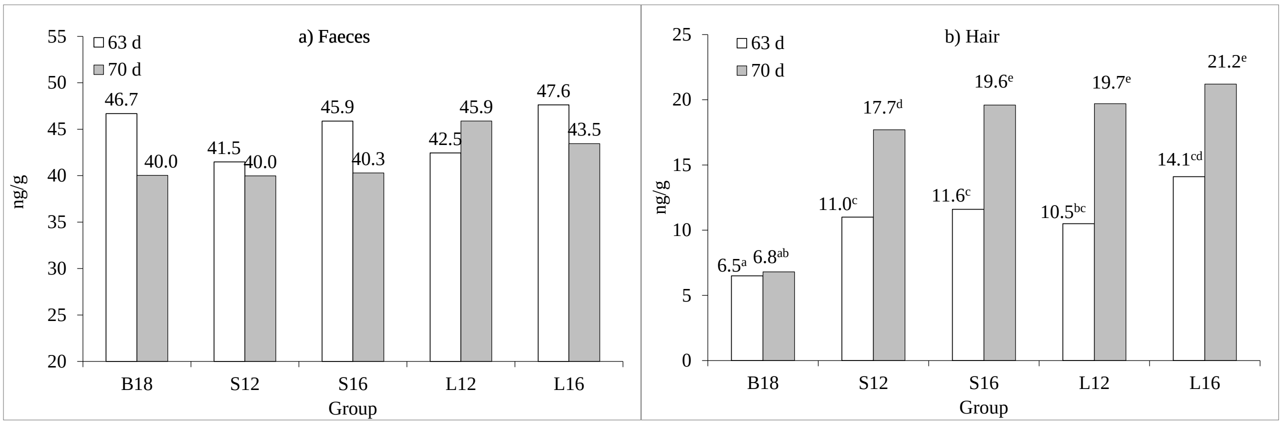


Figure 1.2 Corticosterone levels (ng/g) in hard faeces (a) and hair (b) at 63 and 70 d of age in rabbits reared in bicellular cages (B18), small collective pens at 12 rabbits/m^2 (S12) and 16 rabbits/m^2 (S16), and large collective pens at 12 rabbits/m^2 (L12) and 16 rabbits/m^2 (L16). [Means without a common superscript letter differ, P<0.05]

Discussion

Effect of the housing system: cages vs. pens

Rabbits reared for meat production may be kept in wire net cages in pairs (Italy, Hungary) or in small groups of 4-6 rabbits (France, Spain, Portugal, USA). Nevertheless, alternative systems based on larger cages or pens with large groups of animals are required to permit wide repertoires of movements and behaviours, therefore fulfilling animal welfare needs (EFSA, 2005).

Generally speaking and consistent with our results, the growth performance of rabbits housed in large groups in cages (Szendrő et al., 2009b) or in pens (Lambertini et al., 2001; Martrenchar et al., 2001; Postollec et al., 2008; Princz et al., 2009) is worse compared with rabbits kept in bicellular cages or in small-group cages (≤8 rabbits). However, rabbits kept in conventional wire-net cages in small groups (from 2 to 8 rabbits) show similar growth rates and carcass and meat quality traits (Mirabito et al., 1999; Verga et al., 2004).

The better performance of the rabbits in the bicellular and small-group cages compared with the rabbits in large-group cages may be a result of the ability of the latter to express behaviours other than feeding. Higher competition is likely to occur within large groups of animals, both to establish hierarchies and to gain access to feeders, which may reduce feed intake and, therefore, the growth of the animals. The lower performance of rabbits in pens compared with those in cages may also depend on the type of floor, which may increase the movement of the rabbits inside the pen looking for a more suitable place, as it could have happened when a bed of straw was used (Dal Bosco et al., 2002; Trocino et al., 2008), or which could be unsuitable for rabbit movement and displacements (e.g., a slippery surface), as happened with the wooden slatted floor of the pens used in the present trial. Finally, the design of cages and pens (roofed and open-top, respectively) as well as the materials they were made of (wire net and wooden walls) may have partly accounted for the different results making more complicate the interpretation of the results obtained.

Housing in bicellular cage is recognized as a cause of poor welfare in rabbits reared for meat production due to movement constraints and the lack of environmental and social interactions (EFSA, 2005; Szendrő and Dalle Zotte, 2011). Whether these conditions affect fear and stress levels in animals is not yet clear (Trocino et al., 2013; Verga et al., 2007).

In our trial, rabbits housed in bicellular cages exhibited poorer behavioural patterns because rabbits were not allowed to run, rear or hop, due to the small available surface and the low height of the cages. Previously, authors observed that rabbits kept collectively performed more activities and rested less than rabbits in bicellular cages (Princz et al., 2008a). However, in our study, rabbits in collective pens rested for a longer time. We hypothesise that, especially during the first weeks of the trial, the rabbits were afraid to move across the slatted wooden floor of the collective pens because of the large space between slats (3 cm). These limitations likely led to decreased time spent eating and allo-grooming compared to rabbits raised in bicellular cages.

The body position assumed during resting (more time in a crouched position in collective pens than in bicellular cages) in the first period of observation (52 d) further supports this hypothesis: the crouched position could have been perceived as safer on the wooden slatted floor, with a lower risk of falling into the spaces among slats. On the other hand, at later observations (73 d of age), the very low time spent in resting in a stretched position by rabbits in bicellular cages (0.87%) has likely to be ascribed to the space limits of the bicellular cage itself.

As a consequence of experience or environment, animals may show different attitudes both towards humans (tonic immobility test) and towards an unknown environment (open field test).

In our trial, rabbits kept in collective pens displayed less fear (meaning a lower percentage of rabbits were sensitive to immobility) towards humans in the tonic immobility test, whereas they were less prone to movement and less curious towards a new environment during the open field test compared to rabbits in bicellular cages.

The congruence of rabbit behaviours during the tonic immobility test and the open field test has not been proven definitively. In fact, in contrast with our results, previous studies reported that rabbits in bicellular and small collective cages (9 animals per group) had similar behaviours towards humans and were less fearful in the tonic immobility test compared to rabbits kept in individual cages (Trocino et al., 2013). Rabbits kept collectively were reported as more bold during the open field test compared with rabbits kept in individual or bicellular cages (Schepers et al., 2009; Trocino et al., 2013). This finding was partly related to social isolation, the need for group reinstatement and curiosity, similar to what has been described for birds and sheep (Forkman et al., 2007).

In the conditions of our trial, the possibility of expressing wider social behaviours with more conspecifics in the collective pens seems to have improved animals reaction toward humans. On the other hand, the fearful reaction of these rabbits towards a new environment may be explained by the negative experience they had when moving on the wooden slatted floor of their pens, especially at an early age (post-weaning period), or by the stress induced by the social isolation during the open field test.

The higher levels of hair corticosterone measured in the rabbits reared in collective pens compared to those kept in bicellular cages may also be a signal for the more stressful conditions under rabbits housing in collective pens. Whether this stress came from the housing conditions *per se* (bicellular cages vs. collective pens) or could be attributed to other factors, such as the difficulty of establishing a stable social hierarchy due to the high number of conspecifics, needs to be elucidated. The significant interaction between the housing system and the age of the animals, with hair corticosterone concentrations increasing from 63 to 70 d of age only in rabbits kept in collective pens, suggests that behaviours associated with establishing social hierarchy or oncoming sexual maturation (Szendrő et al., 2009b) could have an effect on glucocorticoid levels. This trend could be even accentuated in the actual genetic lines selected for fast growth rate (Pascual et al., 2013).

Hair cortisol concentrations have been used as an index of chronic stress in various animal species. To our knowledge, little information is available about these measurements in rabbits (Comin et al., 2012). In contrast with our results, glucocorticoid metabolites and corticosterone concentrations in hard faeces have been found to differ among growing rabbits reared in different housing conditions (with or without enrichment), subjected to transport stress (Buijs et al., 2011a), among rabbits of different breeds, between rabbits at different physiological states that were kept in different housing conditions (cage size and presence of plastic foot mat) (Prola et al., 2013), and among wild rabbits after exposure to a stress stimulus (predator odour) (Monclús et al., 2006).

Based on the different accumulation pathways, we could hypothesise that corticosterone in faeces may be useful to measure an acute stress whereas corticosterone in hair may measure a chronic stress in rabbits as it happens in other species (Cook, 2012).

Effect of pen size and stocking density in collective pens

In standard collective cages (for reproduction and fattening), rabbits are reared at different stocking densities in rabbit-producing countries, that is, from 14 to 23 animals/m^2, corresponding to 35 to 52 kg live weight/m^2 at slaughter age depending on the commercial final live weight of rabbits (Trocino and Xiccato, 2006). On the other hand and as mentioned above, EFSA (2005) has given different recommendations.

Scientific information on the key factors affecting productive traits under alternative group housing systems is scarce, and the most appropriate stocking densities or cage dimensions that do not impair productivity or behaviour have not been yet defined. Additionally, the effects of stocking density, group size, and available floor surface may be confounded in collective housing systems, e.g., variable group sizes in cages of the same dimensions or fixed group sizes in cages of different dimensions. In fact, in the present trial, stocking density was changed within pens of the same size by increasing the group size (from 20 to 27 rabbits in the small pens and from 40 to 54 rabbits in the large pens), whereas the pen size was tested, at the same stocking density by doubling the number of rabbits from small to large pens.

In rabbits housed in small groups (up to 10 animals per cage or pen), a reduction in the stocking density from 20-23 to 15-16 rabbits/m^2 significantly improved growth performance (Aubret and Duperray, 1992). In contrast, a further reduction of stocking density from 16 to 12 rabbits/m^2 did not modify growth performance over the entire span of the trial (Princz et al., 2008b; Trocino et al., 2006, 2008).

In most cases, in rabbits housed in rather large groups (up to 64 animals per cage or pen), the reduction of stocking density (15-16 to 8-10 rabbits/m^2) did not affect growth rate and final weight (Lambertini et al., 2001; Szendrő et al., 2009b; present trial), with the exception of the study of Di Meo et al. (2003).

Determining the optimal pen size is far more difficult than the identification of a suitable stocking density for group-housed rabbits. In the present trial, pen size was tested at the same stocking density; in this way, rabbits in large pens had the same individual surface area but a larger total surface area available for movement compared to rabbits in small pens. We found that growth performance was not modified by pen dimensions, which confirms previous results. In fact, Szendrő et al. (2009b) did not find significant differences in growth performance when cage or pen size (and group size) increased from 0.12 m^2 (with 2 rabbits) to 0.50 m^2 (6-8 rabbits) to 0.86 m^2 (10-13 rabbits) to 1.72 m^2 (20-26 rabbits); only the fore part proportion of the carcass increased and the perirenal fat proportion decreased as cage dimensions and group size increased. Postollec et al. (2006) also did not find differences in growth performance or carcass traits when cage size (and group size) was increased from 0.39 m^2 (cages with 6 rabbits) to 0.66 m^2 (small pens with 10 rabbits) to 3.67 m^2 (large pens with 50 rabbits).

In our study, the differences in behaviour, reactivity and stress levels according to pen size and stocking density within collective pens were rather scarce. In small pens, rabbits rested less and spent more time performing other activities (eating, self-grooming, sniffing) in comparison with rabbits kept in large pens. Other authors did not find substantial changes in the budget time of rabbits housed at different pen/cage sizes at the same stocking density (15 animals/m^2) (Martrenchar et al., 2001; Postollec et al., 2006, 2008) or at different stocking densities (Buijs et al., 2011b). Only the frequencies of runs, hops and consecutive hops were significantly higher in rabbits kept in large pens (50 rabbits) compared with rabbits in small pens or standard cages (6-10 rabbits) (Martrenchar et al., 2001; Postollec et al., 2006). These results may indicate that rabbits have specific requirements for a total available surface on which to perform some typical activities, rather than a minimum individual space requirement.

In our conditions, rabbits rested more time in the crouched position in the large pens compared to the small pens and especially at early ages compared to later observations (92.4% and 86.8% of resting time in large and small pens, at 52 d of age; 97.3% and 95.7% of resting time in large and small pens at 73 d of age); this result is difficult to explain and deserves further investigation to assess the real space requirements by rabbits. Buijs et al. (2011b) reported that growing rabbits showed increased sternal lying (i.e. abdomen in contact with the floor, without specifying the position of the fore and hind legs) with decreasing space allowance (same group size in pens of decreasing size). This lying was considered a filling behaviour, rather than comfort behaviour.

The small differences in behaviour among the rabbits kept collectively were not associated with changes in fear or stress levels due to the pen size or the stocking density. This change has been previously reported by other authors using the tonic immobility and open field tests (Trocino et al., 2004, 2008; Zucca et al., 2012) or measuring glucorticoid metabolite concentrations in rabbit faeces (Buijs et al., 2011a). For rabbits kept in groups under alternative housing conditions (free range on ground), the reduction of stocking density from 16 to 12 rabbits/m^2 moved the reactivity of rabbits during the open field test towards a more active behaviour (Verga et al., 1994).

Effects of rabbit age

Rabbits spent most of their time resting in both a wild environment (with variations depending on the vegetation structure and food availability) (Kolb, 1986; Lombardi et al., 2007) and in farm conditions (Princz et al., 2008a; Trocino et al., 2013; present trial). Under farm conditions, the main activities are resting, self-grooming, and feeding regardless of the housing system used or rabbit age (Buijs et al., 2011b; Dal Bosco et al., 2002; Trocino et al., 2008, 2013). Few measurements change with increasing age. Feeding time decreases and grooming increases (Martrenchar et al., 2001; Morisse et al., 1999; Trocino et al., 2013). There is also a higher frequency of running and hopping (Trocino et al., 2013; present trial), which may be associated with the appearance of aggressive interactions among animals. In the present trial, as the rabbits aged, the prevalence of the crouched position during resting further increased. Whether the crouched or the stretched position is the most comfortable for the rabbits is difficult to assess; we can state that the crouched position is preferred based on its prevalence during resting at all ages.

In the tonic immobility test, the duration of immobility decreased from 55 to 75 d of age in rabbits both in bicellular cages and collective systems, proving a reduction of the fear level towards humans. Differences according to pen size or stocking density were detected only in younger rabbits and were no longer presented a few days before slaughter. In the open field test, the lower attitude of animals to move in a new environment at older ages mainly depended on the differences in behaviour of rabbits reared collectively.

We avoided unbiased responses due to habituation of the animals to the test. In previous trials where the reactivity tests repeated on the same animals at two ages, a reduction of the sensitivity of rabbits to the tonic immobility test and a decrease in the latency time before entering the open-field arena had been measured in rabbits at the second tests (Trocino et al., 2013; Xiccato et al., 1999). Therefore, these results cannot be attributed to increased age or to the habituation of the animals to the tests. The handling effect would reduce fear toward humans during the immobility or contact tests (Csatádi et al., 2005; Verwer et al., 2009).

Conclusions

Regardless of stocking density (12 to 16 animals/m^2) or pen/group size (small: 1.68 m^2 and 20-27 rabbits; large: 3.36 m^2 and 40-54 rabbits), rearing rabbits in groups in collective pens impaired growth performance compared to rabbits kept in bicellular cages. Behaviour, reactivity and stress levels were different in rabbits kept in bicellular wire-net cages with tops compared with those in collective open-topped pens with wooden floors; however, the best conditions for rabbit welfare have not been definitively demonstrated. In fact, rabbits in pens displayed a more complete behavioural pattern, despite resting more; they were bolder toward humans, more fearful in new environments and exhibited higher hair corticosterone concentrations. Social contacts in the collective system may account for the improved response to humans, whereas certain environmental constraints (unsuitable floor in pens) or social conditions (isolation during the reactivity test) may explain the negative response to a new environmental situation. In the tested conditions, variation in pen size and stocking density within the collective housing systems exerted only weak effects on a small number of behavioural traits.

Trial No. 2:

Effect of floor type, stocking density, slaughter age, and gender on welfare and productivity of growing rabbits housed in collective pens

The productive results of the Trial No.2 have been published in:

Trocino A., Filiou E., Tazzoli M., Birolo M., Zuffellato A., Xiccato G. 2015. Effect of floor type, stocking density, slaughter age and gender on productive and qualitative traits of rabbits reared in collective pens. Animal. http://dx.doi.org/10.1017/S1751731114003188.

Introduction

Currently in Europe, the majority of rabbit meat production is obtained from rabbits reared in groups of two (the Italian and Hungarian systems) or 4-6 animals (the French and Spanish systems) in wire net cages at stocking densities of approximately 16-18 rabbits/m^2 (Trocino and Xiccato, 2006). These conditions do not permit rabbits to fully express their species-specific behaviours, such as rearing, hopping, running, allo-grooming, etc. (Szendrő and Dalle Zotte, 2011; Verga et al., 2007). Society call for welfare-friendly rearing techniques has created a demand for the application of housing systems alternative to those largely presented in the Mediterranean countries (i.e. main rabbit meat producing countries). Northern countries are going to definitively ban the cage rearing of rabbits, but technical protocols and equipment requirements for pen housing are still missing or being defined before being safely acquired by all producing countries and farmers.

In collective pen systems with large group sizes (>10 rabbits/pen), the risks of aggression among animals and distress due to hierarchy establishment are increased, particularly at later ages and during the approach to sexual maturity (Lambertini et al., 2005; Szendrő and Dalle Zotte, 2011) and some differences between males and femals may be expected basing on how hierarchies may be established (Schololaut et al., 2013; Vervaecke et al., 2010). Pen housing might also result in impaired growth performance and meat quality traits compared with conventional small-group (Dalle Zotte et al., 2009b; Maertens and Van Oeckel, 2001; Princz et al., 2009). EFSA (2005) established 16 rabbits/m^2 as a “safe” stocking density from the perspective of both rabbit performance and animal welfare and being mostly based on literature on cage housing. The benefit from further reducing stocking density (≤16 animals/m^2) with alternative pen housing systems have not yet been definitively identified (Lambertini et al., 2001; Szendrő and Dalle Zotte, 2011).

Few differences have been reported in the performance and behaviours of rabbits maintained on different types of floor (e.g., plastic, steel nets or slats) compared with the standard wire-net floor in cages and pens (Petersen et al., 2000; Princz et al., 2008a and 2009; Trocino et al., 2008). Although alternatives to the latter standard solution have been proposed to benefit welfare issues, unsuitable floors (e.g., straw-bedded floors) in pen-housed rabbits have been proven to jeopardise both growth performance and animal welfare itself (Dal Bosco et al., 2002; Morisse et al., 1999).

The goal of the present study was to identify how the type of floor (wooden slatted floor vs. plastic slatted floor), the stocking density (12 vs. 16 animals/m^2), and the slaughter age (76 vs. 83 d) of rabbits housed in collective pens in large groups (20-27 rabbits) could affect their growth performance, behaviour, reactivity and stress level.

Materials and methods

This study was approved by the Ethical Committee for Animal Experimentation of the University of Padova and funded by the University of Padova. All animals were handled according to the principles stated in EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Animals and housing

The trial took place during the months of May-June in an experimental stable with temperatures that varied between 18 and 25ºC. The rabbits were submitted to a natural photoperiod (11-13 h daylight).

At weaning (33 d age), a total of 376 Hyplus rabbits (Hypharm, Groupe Grimaud, Roussay, France) of both genders were selected from healthy litters and monitored from 34 d until the day before slaughter (76 and 83 d of age). The animals were distributed into 16 open-top collective pens (1.20 × 1.40 m, i.e. 1.68 m^2) at two stocking densities (12 and 16 animals/m^2). Half of the pens were equipped with a wooden slatted floor (slat dimensions: 8 × 8 cm; distance between the slats: 3 cm), and the other half were equipped with a plastic slatted floor (dimensions of the grids: 7 cm length and 1 cm width; distance between the grids: 0.7 cm) (Figure 2.1 and 2.2). The sidewalls of the pens (105 cm-height) were composed of wooden material, and the back/front walls were made of galvanised wire net. The study involved the following four experimental groups:

-W12 (wooden slatted floor, 12 animals/m^2): 80 rabbits maintained in 4 pens with 20 animals/pen;

-W16 (wooden slatted floor, 16 animals/m^2): 108 rabbits maintained in 4 pens with 27 animals/pen;

-P12 (plastic slatted floor, 12 animals/m^2): 80 rabbits maintained in 4 pens with 20 animals/pen;

-P16 (plastic slatted floor, 16 animals/m^2): 108 rabbits maintained in 4 pens with 27 animals/pen.

In order to determine the effect of age on productive traits (in terms of feed conversion index and dressing out percentage) and on lesions incidence and reactivity of animals, within each experimental group, half of the animals (two pens) were slaughtered at 76 d of age, and the remaining animals (two pens) were slaughtered at 83 d of age.

(a) (b)

Figure 2.1. Open-top collective pens equipped with wooden (a) and plastic (b) slatted floor, respectively.

(a) (b)

Figure 2.2. Wooden (a) and plastic (b) slatted floor, respectively.

The animals had *ad libitum* access to fresh water through nipple drinkers and to a pelleted diet through feeders for manual distribution. During the first period (from weaning until 61 d of age), a post-weaning diet (dry matter: 89.5%, crude protein: 15.0%, NDF: 35.5%, ADF: 19.2%, and ADL 4.8%) containing coccidiostat was fed to all rabbits. Thereafter until slaughter, a growing diet (dry matter: 89.4%, crude protein: 15.1%, NDF: 34.0%, ADF: 18.3%, and ADL 4.5%) without coccidiostat was provided. The diets were formulated as in De Blas and Mateos (2010).

Growth performance, sanitary status, and lesion recordings

Individual live weight and cage feed intake were recorded once per week. The health status of the rabbits was monitored daily to detect any clinical signs of disease. Before slaughter, all animals were individually examined for the presence of injuries due to bites and grazes on ears and genitals.

In the course of the trial, only five animals died which were reared on wooden slat floor (three from the W12 and two from the W16 groups). The dead animals did not present any clinical signs of diseases and were not submitted to necropsy.

Behavioural evaluation during 24-h

The behaviour of the rabbits was video-recorded and analysed as described for the Trial No. 1 and during 24-h at two ages: at 50-51 d of age (on all the animals) and either at 71-72 d (on all rabbits to be slaughtered at the first age) or at 78-79 d of age (on all rabbits to be slaughtered at the second age).

Reactivity towards man

Tonic immobility test

The tonic immobility test was performed and evaluated as described in the Trial No. 1. Eighty rabbits (5 animals ×16 pens) at 53 d, 40 rabbits (5 animals from the 8 pens of the first slaughter) at 74 d and 40 rabbits (5 animals from the 8 pens of the second slaughter) at 81 d were used. In order to avoid any habituation to the procedure, the animals participated in the test were not the same among the replications of the test.

Human contact test

Reactivity of the rabbits towards man was, also, tested by implementing the human contact test at two ages; at 49 d on all the pens and again either at 70 d on the eight pens of the first slaughter or at 77 d on the remaining eight pens of the second slaughter. For this, an operator unfamiliar to the animals opened each pen and sat on the entrance of it placing his arm on the centre of the pen and a few centimetres above the floor (at the animals withers height). Animals reaction was video-recorded for three minutes and the latency until the first contact with the operator, the number of times rabbits touched or sniffed him and the distribution of time of each contact/sniffing were evaluated (Csatádi et al., 2007; Verwer et al., 2009).

Reactivity towards an unknown environment/stimulus

Open field test

The open field test was performed and evaluated as described in the Trial No. 1. Eighty rabbits (5 animals × 16 pens) at 54 d, 40 rabbits (5 animals from the 8 pens of the first slaughter) at 75 d and 40 rabbits (5 animals from the 8 pens of the second slaughter) at 82 d were used. In order to avoid any habituation to the procedure, the animals participated in the test were not the same among the replications of the test and had not previously participated in the tonic immobility test.

Novel object test

The novel object test was, also, realised at two ages; at 52 d of age on all the cages and again either at 73 d on the eight cages of the first slaughter or at 80 d on the remaining eight cages of the second slaughter. Two different objects were utilized as unknown for the animals. The first was a half-full bottle of water (1.5 lt) anchored by the cap with a chain of grid iron and dropped, simultaneously, from the roof in the centre of each pen until arriving few centimetres above the floor. An external nest for conventional rabbit does cages, made of galvanized iron, was used as a second novel element and put, at the same time, in the centre of each pen.

The test was carried out so that the object used for one pen was different between the two replications of the test and homogeneously distributed among the experimental groups (floor, stocking density and slaughter age). In details, the first replication (at 52 d of age) was performed by inserting the bottle into the first eight pens and the nest into the resting eight pens; whereas, during the second replication one object was used for the first four pens and the other for the resting four pens. Each pen was video-recorded for ten minutes and the total times of contact with the object and the distribution of time of each contact were evaluated (Verwer et al., 2009).

Corticosterone determination in hard faeces and hair

Individual sampling of hard faeces and hair took place twice (immediately before and 24 hours after the tonic immobility test) on the same animals and at two different ages: at 53-54 d of age by 32 rabbits per experimental group and either at 74-75 d by 16 rabbits per experimental group of the first slaughter or at 80-81 d of age, in case of the second slaughter and always by one male and one female per pen. The corticosterone levels in the samples were measured as described in the Trial No.1.

Commercial slaughter

At the first slaughter at 76 days of age, 186 rabbits reared in eight pens (two pens × experimental group) were individually processed in a commercial slaughterhouse. At the second slaughter at 83 days of age, 185 rabbits in the remaining pens (two pens per experimental group) were individually processed. At both ages, the slaughtering took place following the same procedures described in the Trial No. 1.

Statistical analysis

Individual data regarding the initial and final weights and daily growth were analysed by ANOVA with stocking density, type of floor, slaughter age, and gender as factors with pen as a random effect; the PROC MIXED procedure of the Statistical Analysis System (SAS, 2013) was used for this analysis. The pen data for feed intake and feed conversion were analysed by ANOVA with stocking density, type of floor, and slaughter age as factors; the PROC GLM procedure of SAS was used for this analysis. The frequency of injuries was analysed with the CATMOD SAS procedure according to stocking density, type of floor, slaughter age, gender, and the interactions of these factors. Differences between the means with $P \leq 0.05$ were accepted as statistically significant differences.

Behavioural data were analyzed using the PROC GLIMMIX with floor, stocking density, age and their interactions as fixed effects and time of day as a random effect. An underlying Poisson distribution was assumed for data expressed in percentage of the time of observation. Data from the same pen were treated as repeated measures.

Reactivity data (open-field, tonic immobility, human contact and human contact tests) were analyzed using the PROC GLIMMIX with floor, stocking density, age and gender (where possible) as fixed effects and pen as a random effect.

Corticosterone data were analyzed using the PROC MIXED with floor, age, stocking density, time of sampling and gender as factors of variability and pen as a random effect.

Results

Growth performance, sanitary status and lesions

Rearing rabbits on plastic slatted floors rather than wooden floors had a clear positive effect on the production results (Table 2.1), which also implies a positive effect on rabbit welfare conditions. Specifically, the rabbits maintained on the plastic floor exhibited greater daily weight gain (+18% and +11%) and feed intake (+13% and +9%) during both the first and second periods of growth ($P<0.001$); accordingly, their live weights were higher at both 55 d of age (1844 vs. 1702 g) and at slaughter (2795 vs. 2567 g) ($P<0.001$).

No differences in the proportions of rabbits that exhibited injuries due to aggressions at the end of the trial were observed between the rabbits maintained on the plastic and wooden floors (18.6% on average; P>0.05) (Table 2.1).

With the increase of the stocking density daily weight gain and feed intake worsened (40.2 to 38.7 g/d and 123 to 119 g/d, respectively), with a negative impact on the feed conversion index (3.06 to 3.10) and on the live weight at slaughter (2725 to 2637 g) (P<0.001) (Table 2.1). A higher percentage of rabbits with scratches and lesions due to aggression was observed in the pens with 16 animals/m^2 compared with the pens with 12 rabbits/m^2 (26.2 vs. 8.2%, respectively; P<0.001) (Table 2.1).

As the slaughter age increased from 76 to 83 d of age, live weight at slaughter and daily feed intake of the animals significanlty increased (from 2574 to 2788 g and 119 to 123 g/d, respectively), but daily weight gain significanlty decreased (from 40.0 to 38.6 g/d) (0.001<P<0.02). Accordingly, the feed conversion index was impaired with age (Table 2.1).

Gender only affected daily weight gain from weaning to 55 d, which was lower in females than in males (41.2 vs. 42.3%; P=0.02). The occurrence of lesions at slaughter was less obvious in females than males (11.3 vs. 25.8%; P<0.001).

Table 2.1

Rabbits performance from 34 d of age (weaning) to slaughter: effect of floor type, stocking density, slaughter age and gender

	Floor (F)		Stocking density (D)		Slaughter age (A)		Gender (G)		P-value					RSD
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	76 d	83 d	Female	Male	F	D	A	F×A	G	
Number of rabbits	188	183	157	214	186	185	185	186						
Live weight (g)														
At 34 days	895	896	898	894	894	897	901	892	0.93	0.69	0.79	0.76	0.43	96
At 55 days	1844	1702	1785	1761	1770	1775	1766	1781	<0.001	0.15	0.75	0.16	0.32	144
At slaughter	2795	2567	2725	2637	2574	2788	2680	2682	<0.001	<0.01	<0.001	0.13	0.95	214
Daily weight gain(g/d)														
34-55 d	45.2	38.4	42.3	41.3	41.7	41.8	41.2	42.3	<0.001	0.08	0.82	0.07	0.02	4.49
55 d-slaughter	39.0	35.4	38.5	35.9	38.3	36.2	37.5	36.9	<0.001	0.02	0.05	0.26	0.30	5.30
34 d-slaughter[a]	41.9	36.7	40.2	38.7	40.0	38.6	39.2	39.4	<0.001	<0.01	0.01	0.03	0.63	3.89
Daily feed intake[b] (g/d)														
34-55 d	108	96	103	101	102	102	n.e.	n.e.	<0.001	0.14	0.90	0.29	n.e.	1.68
55 d-slaughter	143	131	140	134	136	138	n.e.	n.e.	<0.001	0.01	0.33	0.28	n.e.	3.96
34 d-slaughter	126	115	123	119	119	123	n.e.	n.e.	<0.001	0.01	0.02	0.21	n.e.	2.48
Feed conversion index[b]														
34-55 d	2.39	2.50	2.44	2.45	2.45	2.44	n.e.	n.e.	<0.001	0.50	0.67	0.07	n.e.	0.03
55 d-slaughter	3.67	3.72	3.64	3.65	3.56	3.83	n.e.	n.e.	0.55	0.16	<0.01	0.67	n.e.	0.14
34 d-slaughter	3.03	3.13	3.06	3.10	2.98	3.18	n.e.	n.e.	0.01	0.17	<0.001	0.32	n.e.	0.06
Dressing percentage (%)	61.4	60.9	61.1	61.1	61.0	61.3	61.0	61.3	0.04	0.97	0.12	0.44	0.11	1.45
Lesions at slaughter (%)	20.2	16.9	8.2	26.2	15.0	22.2	11.3	25.8	0.79	<0.001	0.07		<0.001	

[a]Probability of the interaction floor x slaughter age, $P \le 0.05$; daily weight gain: 43.1 g/d and 40.6 g/d in rabbits reared on wooden floor and slaughtered at 76 and 83 d of age; 36.9 g/d and 36.6 g/d in rabbits reared on plastic floor and slaughtered at 76 and 83 d of age
[b]Pen data
n.e., not-estimable

Behaviour during the 24-h period

Behavioural repertoire was different according to the floor type (Table 2.2). Rabbits reared on the wooden slat floor spent more time resting (68.5 vs. 67.0%; P=0.05) and especially in the crouched position (41.3 vs. 35.1%; P<0.001) compared to those reared on the plastic floor. The former animals spent less time allo-grooming (0.18 vs. 0.25%; P=0.05), running (0.01 vs. 0.07%; P<0.01) and biting elements (0.06 vs. 0.14%; P=0.01).

With the increase of the stocking density from 12 up to 16 animals/m^2, rabbits tended to reduce time spent feeding (8.52 vs. 9.0%; P=0.09) and reduced self-grooming (14.9 vs. 16.1%; P<0.001); whereas, time devoted to total resting (68.9 vs. 66.6%; P<0.01) and crouched position during resting (40.8 vs. 35.5%; P<0.001) increased (Table 2.2).

As rabbits were getting older, they diminished time dedicated to feeding and drinking (11.0 to 6.94% and 1.81 to 1.46%; P<0.001, respectively), to self- (16.7 to 14.3%; P<0.001) and allo-grooming (0.27 to 0.16%; P<0.01), to running (0.06 to 0.01%; P<0.02) and moving (1.61 to 1.20%; P<0.001) and to sniffing (5.49 to 3.36%; P<0.001). They performed fewer times hopping and rearing (0.19 to 0.01 and 0.14 to 0.003, respectively; P<0.001) compared to the younger ones. Older animals augmented time spent resting (63.0 to 72.8%; P<0.001) especially with their body crouched (34.7 to 41.8%; P<0.001).

Reactivity towards man

At the tonic immobility test, immobility duration significantly decreased when stocking density increased from 12 to 16 animals/m^2 (from 68.2 to 49.0 s; P<0.01) and when age at the time of testing increased from 53 to 74 or 80 d of age (from 63.7 to 52.4 s; P<0.001) (Table 2.3). Besides, females rested immobile for more time compared to males (60.4 vs. 55.3 s; P<0.001).

During the human contact test (Table 2.4), rabbits on the wooden floor hesitated more to contact the operator compared to those kept on the plastic floor (48.1 vs. 24.0%; P<0.01). Besides, the older rabbits were being less keen on contacting the operator (26.5 vs. 43.5%; P<0.001).

Reactivity towards an unknown environment/stimulus

During the open-field test, rabbits reared on the wooden slat floor tended to be less keen on entering spontaneously into the arena (60.0 vs. 72.5%; P=0.09), they spent less time in moving (26.0 vs. 31.2 s; P=0.03) and passed fewer times by the centre of it (0.48 vs. 2.27; P<0.001) compared to those reared on the plastic floor (Table 2.5).

Table 2.2

Behaviours during video-recording (% of budget time or number of events): effect of floor type, stocking density and age

	Floor (F)		Stocking density (D)		Age (A)[a]		P-value					
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	50-51d	71-72/78-79d	F	D	A	AxF	AxD	FxD
Observations (n)	384	384	384	384	384	384						
Feeding (%)	8.93	8.59	9.0	8.52	11.0	6.94	0.23	0.09	<0.001	0.32	0.001	0.47
Drinking (%)	1.60	1.65	1.66	1.59	1.81	1.46	0.69	0.50	<0.001	0.40	0.52	0.02
Total resting (%)	67.0	68.5	66.6	68.9	63.0	72.8	0.05	<0.01	<0.001	0.38	0.31	0.53
Body stretched (%)	31.9	27.1	31.1	27.8	28.1	30.7	<0.001	<0.001	<0.001	0.08	0.09	<0.01
Body crouched (%)	35.1	41.3	35.5	40.8	34.7	41.8	<0.001	<0.001	<0.001	0.34	0.01	0.07
Self-grooming (%)	15.5	15.4	16.1	14.9	16.7	14.3	0.67	0.001	<0.001	0.69	0.09	0.68
Allo-grooming (%)	0.25	0.18	0.23	0.19	0.27	0.16	0.05	0.29	<0.01	0.10	0.84	0.04
Moving (%)	1.44	1.34	1.43	1.35	1.61	1.20	0.31	0.44	<0.001	0.01	0.14	0.28
Running (%)	0.07	0.01	0.04	0.02	0.06	0.01	<0.01	0.36	0.02	0.61	0.69	0.92
Biting (%)	0.14	0.06	0.10	0.08	0.08	0.11	0.01	0.48	0.22	0.17	0.56	0.02
Sniffing (%)	4.25	4.24	4.49	4.11	5.49	3.16	0.64	0.06	<0.001	0.12	0.30	0.73
Rearing (n)	0.03	0.01	0.02	0.02	0.14	0.003	0.49	0.75	<0.001	0.71	0.98	0.49
Hops (n)	0.07	0.01	0.02	0.04	0.19	0.01	0.06	0.29	<0.001	0.77	0.94	0.37
Aggressions (n)	0.01	0.002	0.005	0.002	0.03	0.0004	0.57	0.71	0.15	0.78	0.82	0.25

[a]Age at the time of video-recording

Table 2.3

Response to the tonic immobility test: effect of gender, floor type, stocking density and age

	Floor (F)		Stocking density (D)		Age (A)[a]		Gender (G)		P-value									
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	53 d	74/80 d	Female	Male	F	D	A	G	AxG	AxF	AxD	GxF	GxD	FxD
Rabbits (n)	80	80	80	80	80	80	87	73										
Sensitive rabbits (%)[b]	93.8	90.0	92.5	91.3	92.5	91.3	89.7	94.5	0.39	0.77	0.77	0.26						
Attempts (n)	1.51	1.46	1.53	1.44	1.54	1.43	1.42	1.55	0.83	0.66	0.62	0.51	0.66	0.88	0.53	0.45	0.99	0.83
Immobility (s)	61.3	54.4	68.2	49.0	63.7	52.4	60.4	55.3	0.30	<0.01	<0.001	<0.001	<0.001	0.15	0.65	0.40	0.38	0.31

[a]Age at the time of video-recording

[b]The percentage of rabbits turned immobile within three attempts

Table 2.4

Response to the human contact test: effect of floor type, stocking density and age

	Floor (F)		Stocking density (D)		Age (A)[a]		P-value					
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	49 d	70/77 d	F	D	A	AxF	AxD	FxD
Pens (n)	16	16	16	16	16	16						
Rabbits in contact (%)[b]												
within 30”	26.4	9.33	13.1	18.9	23.2	10.6	<0.001	0.03	<0.001	<0.001	0.03	0.14
within 1st min	34.2	15.0	18.8	27.3	31.8	16.1	<0.01	0.09	<0.001	<0.01	0.02	0.62
within 2nd min	6.05	2.55	2.31	6.68	6.05	2.54	0.06	0.03	<0.001	0.25	<0.001	0.05
within 3rd min	6.37	4.87	5.51	5.63	4.90	6.33	0.55	0.96	0.11	0.97	0.35	0.26
Totally	48.1	24.0	28.0	41.3	43.5	26.5	<0.01	0.08	<0.001	0.20	<0.001	0.20

[a]Age at the time of video-recording

[b]Rabbits (%) in the pens that contact the operator within the first thirty seconds (0-30”), the first (0-60”), second (61-120”) and third (121-180”) of the human contact test

Table 2.5

Behaviours during the open field test: effect of gender, floor type, stocking density and age

	Floor (F)		Stocking density (D)		Age (A)[a]		Gender (G)		P-value									
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	54 d	75/82 d	Female	Male	F	D	A	G	AxG	AxF	AxD	GxF	GxD	FxD
Rabbits (n)	80	80	80	80	80	80	80	80										
Entered animals[b] (%)	72.5	60.0	73.8	58.8	76.3	56.3	68.8	63.8	0.09	0.04	<0.01	0.50						
Latency (s)	27.8	27.3	26.6	28.7	27.1	28.1	28.0	27.1	0.77	0.19	0.38	0.43	<0.001	0.34	<0.01	0.01	<0.001	0.02
Total displacements (n)	36.3	35.4	37.4	34.4	38.0	33.9	42.2	30.5	0.83	0.53	<0.001	<0.001	<0.001	<0.001	0.01	<0.01	0.35	<0.01
Central displ. (n)	2.27	0.48	1.29	0.84	1.07	1.02	1.60	0.68	<0.001	0.25	0.73	<0.001	0.78	0.03	<0.01	0.04	<0.01	0.14
Exploration (s)	354	392	366	379	380	365	367	379	<0.01	0.33	<0.001	<0.001	0.01	<0.001	0.54	0.15	<0.001	<0.01
Movement (s)	31.2	26.0	30.8	26.3	29.2	27.9	30.4	26.8	0.03	0.07	0.60	0.15	0.57	0.68	0.83	0.96	0.92	0.23
Running (s)	7.04	5.58	6.72	5.85	12.0	3.27	7.22	5.44	0.31	0.60	<0.001	<0.001	<0.001	<0.001	<0.001	0.03	0.22	0.73
Stay still (s)	52.7	35.1	45.7	40.5	35.4	52.3	39.9	46.4	0.02	0.60	<0.001	<0.001	0.03	<0.001	0.39	<0.001	<0.001	0.011
Grooming (s)	4.25	1.94	3.51	2.35	4.42	1.87	3.60	2.29	<0.01	0.13	<0.001	<0.001	<0.001	<0.01	0.10	<0.001	0.46	<0.001
Resting (lying) (s)	1.99	2.76	2.21	2.48	1.07	5.12	3.72	1.47	0.68	0.92	<0.001	<0.001	<0.001	<0.001	<0.001	0.12	<0.001	<0.01
Digging (s)	0.25	0.68	0.46	0.37	0.19	0.88	0.50	0.33	0.08	0.76	<0.001	0.07	0.13	0.34	0.05	0.04	<0.001	0.02
Digs (n)	0.13	0.44	0.26	0.23	0.15	0.38	0.30	0.19	<0.01	0.78	<0.01	0.20	0.21	0.15	0.21	0.40	<0.01	0.02
Hops (n)	0.84	1.57	0.95	1.39	1.81	0.73	1.35	0.97	0.05	0.25	<0.001	0.05	0.52	<0.01	0.03	0.14	0.15	0.09
Alerts (n)	2.32	0.96	1.77	1.26	1.81	1.23	1.72	1.29	<0.01	0.25	<0.01	0.05	0.37	<0.001	<0.01	0.13	0.94	0.010

[a]Age at the time of video-recording

[b]The percentage of rabbits, spontaneously, entered into the arena

Additionally, the rabbits kept on the wooden floor groomed (1.94 vs. 4.25 s; P<0.01) less than rabbits kept on the plastic floor and spent more time cautiously exploring the arena (392 vs. 354 s; P<0.01).

With the increase of the stocking density from 12 to 16 rabbits/m^2, the percentage of rabbits entered spontaneously into the arena within the first 60 seconds decreased (73.8 to 58.8%; P=0.04) and the time spent in movement tended to diminish (30.8 to 26.3 s; P=0.07).

As age increased from 54 to 75 or 82 d of age, the percentage of rabbits entered spontaneously into the arena decreased (76.3 to 56.3%; P<0.001) and a similar trend was recorded for the number of total displacements (38.0 to 33.9), the time spent in cautious exploration (380 to 365 s), running (12.0 to 3.27 s) and grooming (4.42 to 1.87 s). Older rabbits spent more time (0.001<P<0.01) resting (5.12 vs. 1.07 s), standing still (52.3 vs. 35.4 s) and digging (0.88 vs. 0.19 s).

Gender also significantly influenced rabbits behaviour into the arena (0.001<P<0.07): females accomplished more total (42.2 vs. 30.5) and central displacements (1.60 vs. 0.68) and spent more time running (7.22 vs. 5.44 s), grooming (3.60 vs. 2.29 s), resting (3.72 vs. 1.47 s) and digging (0.50 vs. 0.33 s) compared to males. Females also performed more hops (1.35 vs. 0.97) and alerts (1.72 vs. 1.29). Males spent more time in cautious exploring their surrounding (379 vs. 367 s) and standing still (46.4 vs. 39.9 s) compared to females.

During the novel object test, rabbits reared on plastic slat floor (compared to those on the wooden floor) and younger rabbits (compared to older ones) interacted more with the object (87.2 vs. 50.3% and 78.1 vs. 59.4%, respectively; P<0.001) during the whole duration of the test (Table 2.6). The type of object used for the test also influenced rabbits reaction, with the nest being more used (P<0.001).

Corticosterone in hard faeces and hair

The corticosterone level in hard faeces only tended to be higher for the rabbits reared on the wooden slat floor compared to those reared on the plastic one (41.7 vs. 37.8 ng/g; P=0.06), while decreased in older rabbits (40.2 vs. 36.0 ng/g vs. 34.0 ng/g; P<0.001) (Table 2.7).

Time of sampling (before or after performing the tonic immobility test), gender or stocking density did not affect hair or faeces corticosterone levels.

Table 2.6

Response to the novel object test: effect of floor type, stocking density, age and object

	Floor (F)		Stocking density (D)		Age[a] (A)		Object (O)		P-value					
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	52 d	73/80 d	Bottle	Nest	F	D	A	O	AxF	AxD
Pens (n)	16	16	16	16	16	16	16	16						
Number of contacts (%)[b]														
1st min	16.1	9.76	13.8	12.0	15.3	10.5	9.11	16.7	<0.001	0.15	<0.01	<0.001	0.36	0.05
4th min	23.0	13.7	19.4	17.3	20.3	16.3	13.9	22.8	<0.001	0.31	<0.01	<0.001	0.03	<0.001
7th min	25.7	14.0	21.4	18.3	22.3	17.4	14.8	24.9	<0.001	0.16	0.04	<0.001	0.73	0.02
10th min	22.4	12.9	18.7	16.6	20.1	15.2	10.4	24.9	<0.01	0.42	0.01	<0.001	0.25	0.11
Totally	87.2	50.3	73.4	64.1	78.1	59.4	48.2	89.4	<0.001	0.13	<0.001	<0.001	0.15	<0.01

[a]Age at the time of video-recording

[b]Number of contacts the first, fourth, seventh and tenth minute of the test

Table 2.7

Corticosterone levels (ng/g) in hard faeces and hair: effect of gender, floor type, stockind density, sampling time and age

	Floor (F)		Stocking density (D)		Age (A)[b]			Gender (G)		Sampling (S)[a]		P-value					RSD
	Plastic	Wood	12 rabbits/m^2	16 rabbits/m^2	53 d	74 d	80 d	Females	Males	Pre-IT test	Post-IT test	F	D	A	S	G	
Rabbits (n)	64	64	64	64	64	32	32	64	64	64	64						
Hair (ng/g)	10.4	10.5	10.5	10.4	9.9	12.0	9.5	10.3	10.6	10.1	10.8	0.99	0.91	0.24	0.34	0.71	3.97
Faeces (ng/g)	37.8	41.7	40.6	38.8	40.2^B	36.0^A	34.0^A	39.3	40.2	39.9	39.6	0.06	0.36	<0.001	0.84	0.66	7.20

[a]Sampling of hair and faeces before and after performing the tonic immobility test

[b]Age at the time of video-recording

Discussion

Effect of floor type: plastic vs. wooden slat floor

In the previous trial (Trial No. 1), the rabbits reared collectively in the same pens with wooden slatted floor exhibited lower growth performance compared with the rabbits in conventional bicellular cages, and we hypothesized that this effect was due to the type of floor rather than to the rearing system *per se* (i.e., bicellular cages vs. collective pens). The present results confirm this hypothesis. Similarly to the findings described by Petersen et al. (2000), the 3-cm distance between the wooden slats used in our studies is likely too great to safely support the rabbits, particularly the young animals, and this affects rabbit behaviour, reactivity and stress level.

The rabbits reared on the wooden floor were less keen on moving and exploring their pen as they dedicated less of their time in running, hopping and biting elements of the pen and more in resting; clearly, preferring the crouched position with their limbs under their body compared to those reared on the plastic floor. The rabbits on wooden floor were afraid of slipping into the gap among the slats. Other studies did not show important differences on rabbits behavioural repertoire according to different floor types (plastic net vs. wire net or wire net vs. slat) (Princz et al., 2008a; Trocino et al., 2004), but clearly an animal which faces difficulties to move and to procure feed gives, also, poor productivity traits.

In the present study, no effect of floor type on reactivity during this test was noticed. Similarly, Trocino et al. (2004) did not find differences among wire-net or steel slat floors; whereas in the study of Trocino et al. (2008), when rabbits on steel slat were compared with those on plastic slat, wire-net and straw bedded net, only the number of attempt to enter in immobility resulted lower in those reared on the bedded floor.

The results of the Trial No.1 showed that the possibility of expressing wider social behaviours with more conspecifics in the collective pens improves animals reaction toward humans and this reaction is less affected by the housing conditions (e.g. unsuitable floor). In fact, the lower feeling of rabbits to contact man during the human contact test in rabbits housed on a wooden floor compared to those kept on a plastic floor likely depended on the negative experience they had when moving on the wooden slatted floor, rather than on a fearful reaction towards man.

The open-field test is used to investigate animals reactivity when they face a new environmental situation and it is believed that it mimics the risk of predation for those species that in nature are used to hide (Forkman et al., 2007). In the present study, rabbits reared on the wooden floor were more hesitant to enter spontaneously into the arena, they moved, assumed the alert position and displayed themselves less, which could be interpreted as less "bold" behaviours; they explored more their surroundings, which could be interpreted as a "prudent" behaviour (Ferrante et al., 1992; Meijsser et al., 1989; Trocino et al., 2013; Verga et al., 1994). In the study of Trocino et al. (2008) when rabbits reared on different floors were tested with the open-field test, rabbits grown on the uncomfortable floor (straw bedded wire) stood still for more time, and explore the arena less compared to the others, which were considered as "passive" behaviours by the authors confirming the inadequacy of that floor type.

The novel object test is used as a way to understand fear level of animals when they confront with a new stimulus. Irrespective of the age, the floor or object type, in the present study, rabbits demonstrated their interest to contact with the new object within the first minute of the test, whereas this "enthusiasm" was gone after the seventh minute. Also, Hoffman and Basurto (2013) noticed a decrease of the "chinning" behaviour on new objects during a 15 min-period testing, as a result of habituation response. Nevertheless, the animals reared on the wooden slat floor boggled and contacted the new object less than those on plastic floor. Interesting was the higher percentage of rabbits that preferred to get in touch with the nest rather than the bottle. Related studies are not widespread for rabbits.

Based on the behavioural and reactivity results, it can be assumed that floor type generally influenced rabbits stress, as they were "forced" to move discreetly and rest picking their limbs close to their body by the fear of being embedded among the slats. This stress induced did not change their reactivity towards human (tonic immobility test); whereas the same rabbits exhibited a more fearful and less active behaviour when put into a new environment (open field test). Additionally, rabbits from the wooden floor were more afraid to approach both a new person and a new object (human contact and novel object tests).

The foregoing comments are, also, verified by the results of the corticosterone analysis which showed that rabbits reared on the wooden slatted floor had higher concentration of corticosterone in their faeces. Nevertheless, corticosterone analysis in hair did not give any significant result. This could have happened because of inappropriateness of time of sampling (age of animals). Hence, the first sampling happened at 53 d of age when regularly are use to lose hair; whereas, the second sampling came about a period less stressful for rabbits (before slaughter).

Effect of stocking density: 12 vs. 16 rabbits/m^2

At the end of the trial, the slaughter weights per unit of pen surface were 32.7 kg/m^2 and 42.2 kg/m^2 in the pens with 12 and 16 rabbits/m^2, respectively. The latter figure was somewhat higher than the recommended value (40 kg/m^2; EFSA, 2005), above which both growth performance and behaviour pattern might exhibit some weakness when rabbits are reared in cages (Maertens and De Groote, 1984; Morisse and Maurice, 1997). Indeed, at the end of our trial, a higher percentage of rabbits with scratches and lesions due to aggression was observed in the pens with 16 animals/m^2 compared with the pens with 12 rabbits/m^2, which evidences impairments of the general welfare conditions. The available surface likely became a limiting factor during the last week of the trial under the Italian conditions, which use rather late slaughtering (>75 d of age). In fact, until 9 weeks of age, the increase of space allowance did not affect behaviour and use of space in rabbits kept in cages (Buijs et al., 2011b). Accordingly, the availability of a larger functional space to be used by all rabbits in pens compared to cages would permit to overpass the above mentioned threshold recommended by EFSA (2005) without great negative consequences when rabbits are kept in pens, provided that slaughtering occurs within 10 weeks of age. The possibility of maintaining the same production level per unit of surface even in alternative pen housing systems is crucial for the economic sustainability of rabbit farmers and for a safe transition from classical cages to pen systems in main rabbit meat producing countries.

Stocking density affected behavioural budget time of rabbits in the present trial. Hence, increasing stocking density from 12 to 16 animals/m^2 rabbits increased resting time with their body crouched and reduced feeding and self-grooming. Similar results were obtained by Morisse and Maurice (1997); resting was increased and feeding decreased when stocking density increased from 15.3 to 23 rabbits/m^2. Contrary, Trocino et al. (2004) did not observe any significant effect of stocking density on rabbits behaviour.

Stocking density, only slightly, influenced rabbits response during the tonic immobility and open-field tests. Also other studies confirmed negligible effect of stocking density to rabbits reactivity during these tests, when same stocking densities were under investigation (Trocino et al., 2004, 2008). Ferrante et al. (1997) found a reduction in time spent in movement during the open-field test when stocking density increased from 12 to 17 rabbits/m^2 and authors attributed it to the more stressful environmental condition (17 rabbits/m^2) which was reducing rabbits interaction with their surroundings.

Effect of age

The deterioration of the productivity results with inscreasing slaughter age when rabbits are reared in large groups may be more evident than in conventionally rearing because of the simultaneous increase of injured animals due to elevated aggression, as happened in the present study when rabbits were approaching sexual maturity (age at the second slaughter). Alike with these results, also other authors noticed an increase of body lesions when age increased (Rommers and Meijerhof, 1998; Szendrő et al., 2009b).

In accordance with other studies (Dal Bosco et al., 2002; Martrenchar et al., 2001; Morisse et al., 1999; Morisse and Maurice, 1997; Trocino et al., 2008), rabbits passed most of their time resting, self-grooming, feeding and drinking and, irrespective of their age, they were more active during dark period (Buijs et al., 2011b; Postollec et al., 2006 and 2008; Trocino et al., 2013). In the present study, during the ethogram evaluation, it was revealed that increasing age animals reduced time spent in their nutrition, manipulation of their surroundings and typical behaviours (rearing, hopping); whereas, they increased time devoted in resting with body crouched.

Time spent immobile during the tonic immobility test decreased in older rabbits. Also, with the increase of age rabbits curiosity towards man and object declined during the human contact and object tests, respectively. In the case of the present trial, reactivity tests were applied on different animals at the two ages, in order to evaluate age effect without messing with eventual habituation of animals to the test. Contrary to the present study, when the tonic immobility test was applied at the same rabbits at two different ages, sensibility of the older rabbits to the test was decreased (Trocino et al., 2013). Of course, it is known that frequent manipulation of the animals reduces fear level towards man during the tonic immobility or human contact test (Csatádi et al., 2005; Verwer et al., 2009).

During the open-field test, older animals were more passive, as already outlined from results of the Trial No. 1: lower percentage of animals entered spontaneously into the arena, they performed less displacements and they decreased the behaviours normally performed when they are calm (grooming); instead, they augmented time standing still (indication of fear). When the test was repeated on the same animals (Trocino et al., 2013; Xiccato et al., 1999) less curiosity was noticed by the more mature rabbits to enter the arena and to move, run or assume the alert position; indicating habituation to the test.

In the conditions of the present trial, the reduction of corticosterone concentration in faeces with animal age may be attributed to a progressive adaptation of the animal to the environment they were living in.

Effect of gender

Commercially, growing rabbits are housed in mixed groups (males and females) due to the little sexual dimorphism and the early age they proceed to slaughter. In fact, in the present study gender did not influence growth performance of rabbits. Overall, the small and non-relevant differences in growth performance do not justify the separation of rabbits by gender when they are maintained in the most common and standard housing conditions (i.e., bicellular cages or small cages with 4-6 rabbits). However, in collective housing systems (i.e., large pens with more than 10 animals), the different degrees of social interactions might justify such separation. Vervaecke et al. (2010) demonstrated that rabbits, even those in cages and in small groups (8 animals), develop hierarchies beginning at 10 weeks of age and that males and females do not develop separate hierarchies. In our conditions, differences in the occurrence of injured animals were significantly dependant on gender (fewer females than males were injured). It is not clear if this result depended on major aggressiveness of the females towards the males or major aggressiveness among the males approaching sexual maturity. In this regard, Di Meo et al. (2003) observed a higher occurrence of injuries in rabbits that were maintained in mixed-sex pens (30 rabbits per pen) compared with rabbits that were maintained in single-sex pens. On the other hand, the percentage of rabbits with injuries was noticeably higher in males than females. Aggressions may be more severe among males and especially when sexual maturity approaches and rabbits are reared in large groups (Bigler and Oester; 1996; Verga et al., 2007).

Rabbits response to the tonic immobility and open-field tests was ambiguous between sexes. In details, females were observed more afraid during the tonic immobility test, being for more time immobile; whereas, during the open-field test they were more active (more time spent in locomotory behaviours), and performed more species-specific behaviours (digging, hopping, alert) than males did so.

Conclusions

Wooden slatted floor with 3-cm distance between slats resulted less suitable for growing rabbits as it worsens productivity and welfare (animals prudent to move and constrained to rest in crouched position). The latter was confirmed also by the less active behaviours of rabbits when they undergone the open-field test and by the higher level of corticosterone in their faeces.

Pen stocking density, slaughter age and gender exerted only minor effects on growth performance, behaviour and reactivity and, from the results obtained, it may be assumed that a stocking density of 16 animals/m^2 and a live weight at slaughter of about 40-42 kg/m^2 are compatible with both productivity and welfare threshold. However, a higher incidence of injuries was observed among the male rabbits, at the higher stocking density and the increased slaughter age. From the perspective of optimising conditions for rearing rabbits in pens and in large groups, the option of separately rearing females and males deserves further investigation.

Trial No.3:

Effect of environmental enrichment and slaughter age on welfare and productivity of growing rabbits housed in collective pens

Introduction

In comparison with the traditional cages, a somewhat reduction of productive performance has been generally described in rabbits kept collectively in alternative systems (Postollec et al., 2006 and 2008; Trial No. 1), in which animals have the possibility of performing more behaviours and fully expressing social activities (Dal Bosco et al., 2002; Trocino et al., 2013). This not necessarily implies that rabbits under collective systems and with large space to use are less stressed compared to those in smaller cages. In fact, high corticosterone levels have been found in rabbits kept in collective systems compared to those in bicellular cages, especially at increasing age (Trial No 1 present thesis). This result could be attributed to the occurrence of aggressive interactions for establishing social ranks in animals approaching sexual maturity, as it happens under wild conditions (Schlolaut et al., 2013). Accordingly, controlling slaughter age and gender composition of animals kept collectively may provide some advantages in collective systems for growing rabbits.

The presence of some enrichment elements may offer animals the possibility to reduce stress level and control aggressions by different means. Among the different types of enrichment, gnawing objects or mirrored surfaces have been widely tested to enrich rabbit behavioural repertoire (Buijs et al., 2011a; Dalle Zotte et al., 2009a; Jones and Phillips, 2005; Princz et al., 2008a; Zucca et al., 2012) but, basing on rabbit behaviour in the wild, also other structures could have an interest. Elevated platforms have been tested in rabbits kept for meat production to provide rabbits with a shelter, as well as, an occasion to perform hops (Postollec et al., 2008; Szendrő et al., 2012a). Besides, the use of hiding boxes has been evaluated in adult rabbits kept for laboratory purposes (Hansen and Berthelsen, 2000; Lidford, 1997) which may be proposed for growing rabbits too. In fact, wild young rabbits after weaning use to spend most of their time huddled together in a burrow or another shelter; they go outside of the burrow for feeding at dusk and down at an intensity and frequency which is conditioned by food availability, weather conditions and predators presence (Schlolaut et al., 2013).

Therefore, the present study aimed at evaluating whether and how the presence of an elevated platform and/or the presence of a hiding tube may affect growth, behaviour, fear and stress levels of rabbits reared collectively and slaughtered at two ages.

Materials and Methods

Animals and housing

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 the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

The trial was performed in a non-air-conditioned stable during the months of March and April. The temperature ranged from 13 to 24°C, and natural photoperiod (11-13 h daylight) was maintained.

A total of 504 rabbits of both genders from the Hyplus crossbred line (Hypharm, Groupe Grimaud, Roussay, France) was moved from a commercial rabbitry to the experimental facilities of the University of Padova and controlled from weaning (33 d of age) until slaughter (at 68 d or 75 d of age). All rabbits were given *ad libitum* access to a commercial diet for growing rabbits.

The rabbits were put into 16 open-top collective pens (1.40 × 1.20 m = 1.68 m^2) with plastic slatted floor (dimensions of the grids: 7 cm length and 1 cm width; distance between the slats equal to 0.7 cm), wooden side walls (105 cm-height), and front and back walls covered by a galvanised wire-net (Figure 3.1). The pens were equipped with 4 feeders (43.5 × 11.5 × 30.0 cm-height) for the manual distribution of feed at the side walls and 4 nipple drinkers on the back side. Half of the pens were equipped (P) or not (W) with a plastic platform (same material of the floor; 50 × 120 cm; 30 cm above the plastic floor) and (within P and W pens) contained (T) or not (N) a plastic tube (ϕ 20 cm; length: 50 cm) firmly positioned at the center of the pens (Figure 3.2). Accordingly, rabbits were assigned to four experimental groups, as detailed below:

- PT, 4 pens with platform and tube; 36 rabbits/pen, 16 rabbits/m^2 (platform area included), 114 rabbits;
- PN, 4 pens with platform and without tube; 36 rabbits/pen, 16 rabbits/m^2 (platform included), 114 rabbits;
- WT, 4 pens without platform and with tube; 27 rabbits/pen, 16 rabbits/m^2, 108 rabbits;
- WN, 4 pens without platform and without tube; 27 rabbits/pen, 16 rabbits/m^2, 108 rabbits.

The rabbits of half of the pens were slaughtered at 68 d and the other half at 75 d of age.

Figure 3.1. Open-top collective pens with plastic slatted floor.

(a) **(b)**

(c) **(d)**

Figure 3.2. The pens without environmental enrichment (a), only with the plastic tube (b), only with the plastic elevated plarform (c), with both of the enrichment elements (d).

Growth performance, sanitary status and body injuries

Individual live weight and pen feed consumption were controlled two times per week. During the trial, sanitary status was monitored on a daily basis to intervent in case of any clinical sign of disease. In the course of the trial, only four animals died: two from the PT, one from the WN and one from the WT group because of gastrointestinal disturbances.The day before each of the two slaughters (at 67 and 74 d of rabbits age) the presence of injuries on genitals and their severity were recorded for every rabbit by using a score table (Table 3.1).

Table 3.1

Injuries score from 0 to 3.

Score	Description
0	No injuries
1	Injuries limited and superficial
2	Injuries evident
3	Wounds or bleeding injuries

Behavioural evaluation during 24-h

The behaviour of the rabbits was video-recorded and analysed as described for the Trial No. 1 in all pens for 24 hours at two ages, 63-64 and 70-71 d of age. Additionally, the following behaviours were also analysed: stretching (stretching the body or the limbs) and caecotrophy (mouth among hind legs and swallowing soft faeces by anus). Two minutes were evaluated each half-hour per each pen. The behaviours were analysed as percentage of the total observed time (feeding, drinking), as percentage of the time that rabbits were visible (time of rabbits under the platform and/or inside the tube was not considered) (behaviours other than feeding and drinking) or as number of events.

Test of reactivity

Open field test

The test of open field was performed as described in the Trial No. 1 and at two ages, 65 and 72 d, on a total of 160 rabbits (20 rabbits × 4 experimental groups × 2 ages). At each age, 10 rabbits per pen were controlled.

Tonic immobility test

The tonic immobility test was performed as described in the Trial No. 1 and at two ages, 66 and 73 d of age, on a total of 160 rabbits (20 rabbits × 4 experimental groups × 2 ages). At each age, 10 rabbits per pen were controlled. The rabbits tested had not been previously submitted to the open field test.

Corticosterone determination in hair

At 66 and 73 d of age and after the tonic immobility test, samples of hair were collected and analysed as described in the Trial No.1. At each age, 48 rabbits were controlled (3 female and 3 male rabbits × 2 pens × 4 experimental groups).

Commercial slaughter

At the first slaughter at 68 d of age, 250 rabbits reared in eight pens (8 pens corresponding to 2 pens × 4 experimental groups) were individually processed in a commercial slaughterhouse. Similarly were slaughtered the rest of the animals (250 rabbits: 8 remaining pens corresponding to 2 pens × 4 experimental groups). At both ages, the slaughter took place following the same procedures of the Trial No. 1.

Statistical analysis

Individual data for the final weights, daily growth rates and dressing percentages were analysed using the PROC MIXED of the Statistical Analysis System (SAS, 2013) with presence/absence of platform, presence/absence of tube, slaughter age, gender and their interaction as factors with pen as a random effect; the PROC MIXED procedure of the was used for this analysis. The pen data for feed intake and feed conversion were analysed by the PROC GLM procedure of SAS with presence/absence of platform, presence/absence of tube, slaughter age as factors.

The data set of behavioural data was analysed by a mixed model in SAS, i.e. PROC GLIMMIX with presence/absence of platform, presence/absence of tube, age and their interactions as fixed effects, time of the day as random effect and with the details already given in the Trial No. 1. The data of the reactivity tests were analysed by PROC GLIMMIX with presence/absence of platform, presence/absence of tube, age, sex and their interactions as fixed effects, and pen as random effect. The data of hair corticosterone levels was analysed by ANOVA using the PROC MIXED (SAS, 2013) with presence/absence of platform, presence/absence of tube, age, sex and their interactions as fixed effects, and pen as random effect.

The data regarding the severity of the injuries were tested for their normality; data not normally distributed were analysed using the PROC NPAR1WAY (SAS, 2013). The frequency of injuries was analysed with the CATMOD SAS procedure according to stocking density, type of floor, slaughter age, gender, and the interactions of these factors.

Results

Growth performance, sanitary status and body injuries

The presence of platforms inside the pens did not affect performance on the whole period, but augmented the percentage of animals with injuries on genitals at the end of the trial (20.6 vs. 11.7% in rabbits kept in pens with platforms compared to those kept in pens without platforms; $P<0.01$) (Table 3.2). Differently, the enrichment of pens with a tube impaired growth rates affecting final live weight at slaughter ($0.001<P<0.01$), but did not affect the occurrence of lesions (Table 3.2).

The increase of the slaughter age increased final live weight (2467 to 2600 g), decreased daily weight gain (39.2 to 43.8 g/d) ($0.001<P<0.01$), and tended to increase the percentage of animals with injuries on genitals (15.6 to 18.0%; $P=0.08$).

The effect of the gender was evident: females showed higher daily weight gain during the first period and, to a lower extent, during the second period which increased final live weight compared to males (2559 vs. 2508 g; $P=0.02$) (Table 3.2). A higher percentage of injured males was observed, especially due to animals showing injuries on genitals (29.3 vs. 4.74%; $P<0.001$) compared to females (Table 3.2).

Behaviour during the 24-h period

In the pens with platforms, rabbits spent 20.6 and 29.4% of their time above and under the platform, respectively (Table 3.3). The time spent feeding was similar, but a significant interaction ($P=0.05$) was measured with the animals age: feeding time was shorter in pens with platforms than in those without platforms (7.93 vs. 8.41%) at 63 d of age in pens while similar at 70 d of age (8.53 vs. 8.21%) (Table 3.3). In the pens with platforms, rabbits rested more with stretched body (33.7 vs. 31.4%) compared with rabbits kept in pens without platform (% of visible activity); besides the former rabbits spent more time in the rearing position (0.13 vs.

0.06%) or licking and biting elements of their pen (0.37 vs. 0.21%) and less time in self-grooming (14.7 vs. 16.1%), allo-grooming (0.82 vs. 1.30%), moving (0.72 vs. 0.92%), inactive (0.12 vs. 0.21%) and sniffing (4.09 vs. 4.75%) ($0.001<P<0.05$) (Table 3.4).

Table 3.2

Rabbits performance from 33 d of age (weaning) to slaughter (at 68 or 75 d of age): effect of environmental enrichment, age, gender and their interactions

	Platform (P)		Tube (T)		Age (A)		Gender (G)		P-value							RSD
	with	without	with	without	68 d	75 d	Males	Females	P	T	A	PxT	PxA	TxA	G	
Rabbits (n)	286	214	249	251	250	250	246	254								
Live weight (g)[a]																
At 33 d	975	975	976	974	-	-	976	974	0.97	0.85	-	0.97	-	-	0.85	120
At slaughter	2567	2500	2467	2600	2467	2600	2508	2559	0.13	<0.01	<0.01	0.87	0.22	0.02	0.02	241
Daily weight gain (g/d)(33 d-slaughter)[a, b]	42.4	40.7	39.7	43.4	43.8	39.2	40.8	42.2	0.09	<0.001	<0.001	0.92	0.12	<0.01	<0.01	5.1
Daily feed intake (g/d)[c]	147	147	145	149	144	150	-	-	0.99	0.07	<0.01	0.16	0.68	0.34	-	3.9
Dressing percentage (%)	59.6	59.4	59.4	59.6	58.8	60.2	59.6	59.4	0.35	0.58	<0.001	0.75	0.96	0.04	0.25	1.6
Rabbits with lesions on genitals (%)	20.6	11.7	18.9	14.7	15.6	18.0	29.3	4.74	<0.01	0.34	0.08	0.09	0.09	0.26	<0.001	
Severity of the lesions[d]	0.78	0.70	0.83	0.75	0.95	0.53	0.90	0.59	0.89	0.01	<0.001	-	-	-	<0.001	

[a]Individual data

[b]Probability of the interaction TxA, P<0.01; daily weight gain: 43.3 g/d and 36.0 g/d in rabbits kept in pens with tube and slaughtered at 68 d and 75 d of age, and 44.4 g/d and 42.4 g/d in in rabbits kept in pens without tube and slaughtered at 68 d and 75 d of age, respectively

[c]Pen data

[d]Overall score from 0 to 3. Non-parametric analysis of variance which does not allow testing the interactions with PROC NPAR1WAY

The presence of the tube inside the pens did not affect budget time of rabbits which spent 2.44% of their time inside tube (Table 3.3).

The increase of the age from 63 to 70 d significantly affected the budget time: rabbits spent more time above the platform (9.7 to 11.6% of total observed time) (Table 3.3); they increased time spent self-grooming (13.4 to 17.7% of visible time) and sniffing (3.71 to 5.24%) at the expenses of total resting (66.5 to 60.6%), and the time spent resting in crouched position (27.8 to 33.2%) at the expenses of resting in a stretched position (38.7 to 27.4%); the number of hops decreased (0.12 to 0.02) (Table 3.4).

Rabbits were on platform more frequently the time of the highest activity and they preferred to stay under platform the period of resting (Figures 3.3 and 3.4). Irrespective of the experimental group, time spent feeding and moving were negatively correlated with time spent resting; time spent self-grooming had a more uniform distribution showing only an increase during early morning (from 4.00 to 10.00 h) (Figure 3.5). Time that visible rabbits spent performing caecotrophy indicated two peaks, in the morning (from 06.00 to 10.00 h) and in the afternoon (from 15.00 to 17.00 h) (Figure 3.6). Regarding the usage of the tube within 24 hours, a homogeneous distribution was evidenced (Figure 3.7). However, in the pens with only tube, rabbits were using it more than those in pens with both tube and platform (Figure 3.7).

Table 3.3

Behaviours during video-recording at 63 and 70 d of age (% of the total time of observations): effect of environmental enrichment, age and their interactions

	Platform (P)		Tube (T)		Age (A)		P-value						
	with	without	with	without	63 d	70 d	P	T	A	PxT	PxA	TxA	PxTxA
Observations (n)	576	576	576	576	768	384							
Above platform[a] (%)	20.6	0.00	10.6	10.0	9.69	11.6	-	-	-	-	-	-	-
Under platform[a] (%)	29.4	0.00	14.9	14.5	15.2	13.6	-	-	-	-	-	-	-
Inside tube[a] (%)	0.13	2.32	2.44	0.00	1.13	1.42	-	-	-	-	-	-	-
Visible activity[b] (%)	71.0	97.5	82.0	84.5	82.4	84.1	<0.001	<0.001	<0.01	<0.01	<0.001	0.87	0.26
Feeding (%)	8.22	8.31	8.05	8.50	8.17	8.37	0.30	0.83	0.28	0.42	0.05	0.12	0.88

[a]Data not suitable for analysis by PROC GLIMMIX

[b]Time during which rabbits were visible; it includes the time spentabove floor and above platform and it excludes the time spent under platform and/or inside tube

Table 3.4

Behaviours during video-recording at 63 and 70 d of age (% of visible activity):effect of environmental enrichment, age and their interactions

	Platform (P)		Tube (T)		Age (A)		P-value					
	with	without	with	without	63 d	70 d	P	T	A	PxT	PxA	TxA
Observations (n)	576	576	576	576	768	384						
Total resting (%)	63.6	63.4	63.5	63.5	66.5	60.6	0.85	0.98	<0.001	0.21	0.66	0.38
With crouched body (%)	29.5	31.3	30.5	30.3	27.8	33.2	0.02	0.88	<0.001	0.93	<0.001	0.81
With stretched body (%)	33.7	31.4	32.3	32.8	38.7	27.4	0.01	0.65	<0.001	0.35	<0.001	0.93
Feeding (%)	11.3	8.49	9.67	9.89	9.72	9.84	<0.001	0.64	0.59	0.56	0.14	0.16
Drinking (%)	2.34	2.24	2.18	2.40	2.30	2.20	0.56	0.20	0.08	0.84	0.48	0.48
Self-grooming (%)	14.7	16.1	15.7	15.1	13.4	17.7	0.01	0.27	<0.001	0.99	<0.001	0.06
Allo-grooming (%)	0.82	1.30	1.07	1.00	0.98	1.09	<0.001	0.52	0.12	0.13	0.17	<0.01
Sniffing (%)	4.09	4.75	4.40	4.42	3.71	5.24	0.05	0.97	<0.001	0.02	<0.01	<0.001
Moving (%)	0.72	0.92	0.84	0.79	0.82	0.81	<0.01	0.50	0.85	<0.01	0.75	0.49
Other behaviours:	0.94	1.28	1.02	1.19	1.23	0.98	0.01	0.23	0.001	0.56	0.01	<0.001
Licking, biting (%)	0.37	0.21	0.25	0.31	0.31	0.25	<0.01	0.27	0.08	0.93	0.45	0.05
Sitting (inactive) (%)	0.12	0.21	0.16	0.16	0.27	0.09	<0.01	0.86	<0.001	0.28	0.26	0.99
Stretching (%)	0.03	0.03	0.03	0.03	0.03	0.03	0.99	0.81	0.81	0.38	0.94	0.88
Alerts (%)	0.13	0.06	0.07	0.11	0.09	0.09	<0.01	0.05	0.72	0.89	0.72	0.36
Caecotrophy (%)	0.14	0.12	0.17	0.09	0.19	0.08	0.65	0.11	<0.001	0.03	0.05	0.04
Hops (n)	0.05	0.06	0.05	0.06	0.12	0.02	0.83	0.26	<0.001	0.23	0.91	0.23
Jumps from/to platform/tube[a] (n)	0.93	0.04	0.60	0.36	0.49	0.48	-	-	-	-	-	-
Aggressions (n)	0.003	0.010	0.007	0.004	0.007	0.004	0.34	0.64	0.61	0.65	0.93	0.83
Alerts (n)	0.17	0.10	0.12	0.14	0.17	0.10	<0.01	0.39	<0.01	0.91	0.84	0.39

[a]Data not suitable for analysis by PROC GLIMMIX

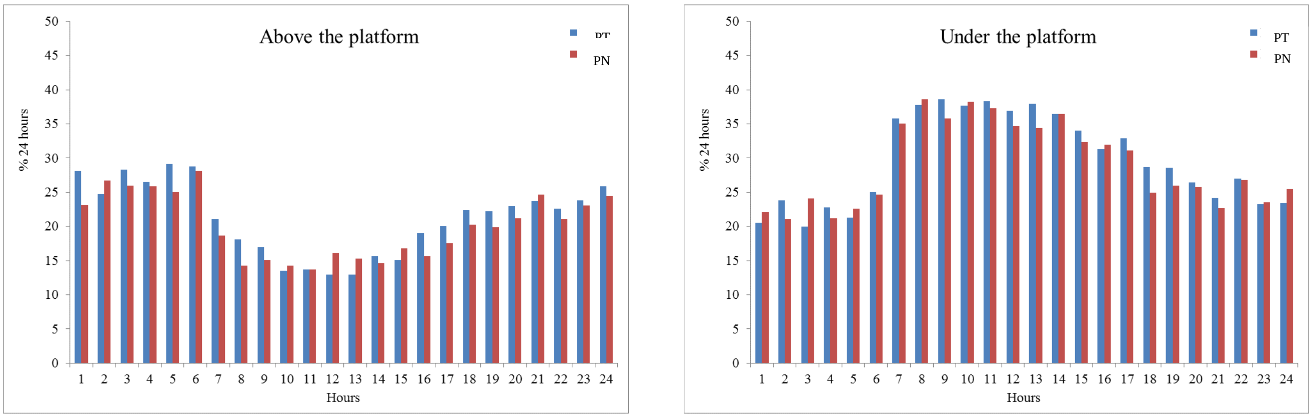


Figure 3.3. Time rabbits spent above and under the platform within 24-h (average of the observations at 63 and 70 d of age for all the rabbits); PT: platform and with tube, PN: platform and no tube

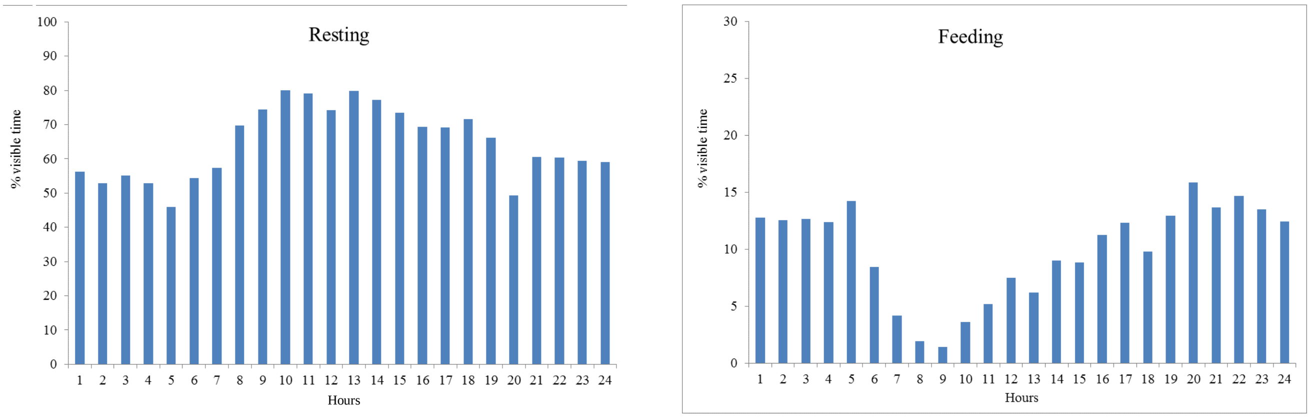


Figure 3.4. Time rabbits spent in resting and feeding within 24-h (average of the observations at 63 and 70 d of age for all the rabbits)

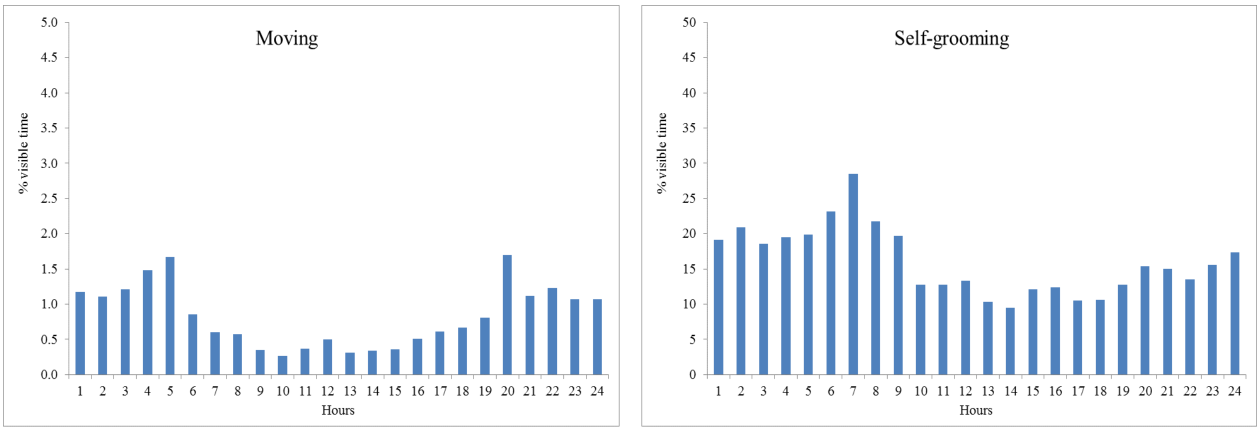


Figure 3.5. Time rabbits spent in moving and self-grooming within 24-h (average of the observations at 63 and 70 d of age for all the rabbits)

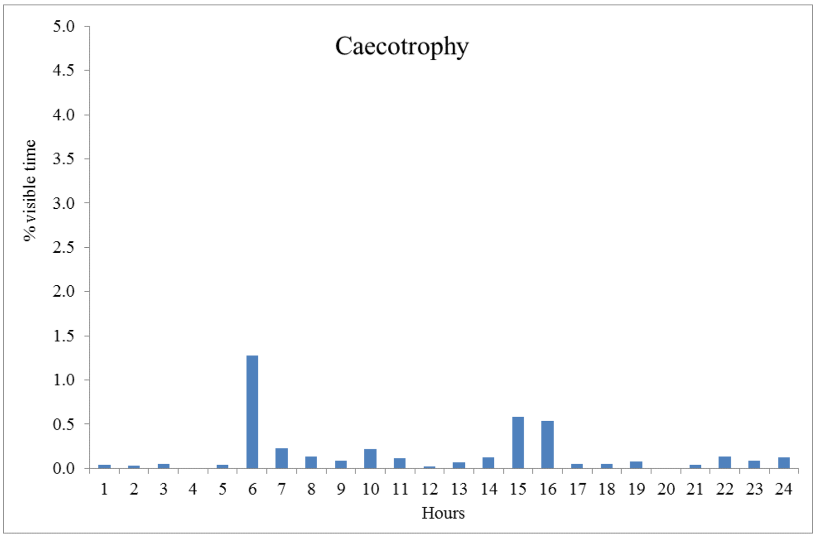


Figure 3.6. Time rabbits spent in caecotrophy within 24-h (average of the observations at 63 and 70 d of age for all the rabbits)

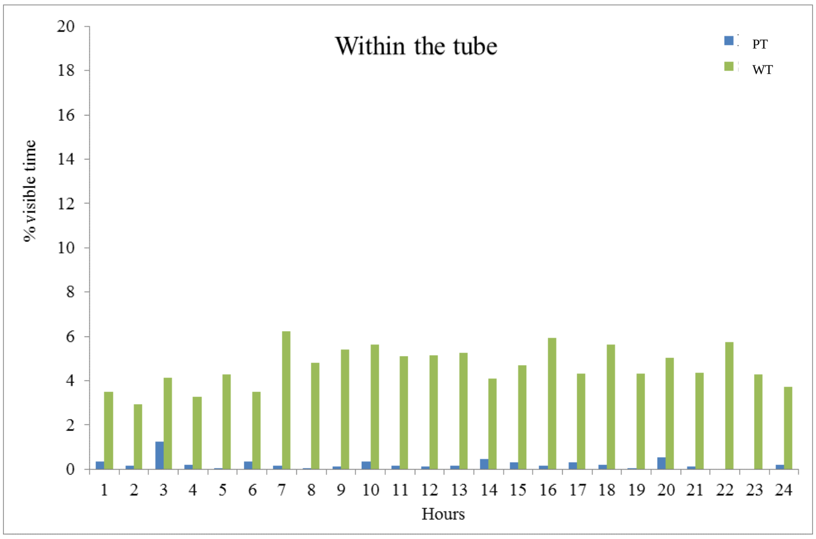


Figure 3.7. Time rabbits spent in the tube within 24-h (average of the observations at 63 and 70 d of age for all the rabbits); PT: platform and tube; WT: without platform and with tube

Tests of reactivity

None of the factors affected rabbits reactivity during the tonic immobility test (Table 3.5). Rabbits reactivity during the open field test changed with the animals age ($0.001<P<0.09$) and gender ($P<0.001$) (Table 3.6). Older rabbits (72 vs. 65 d) crossed a higher number of squares (53.9 vs. 48.2) and spent more time cautiously exploring the environment (biting: 11.7 vs. 2.87 s; exploration: 534 vs. 511 s) and they crossed less times the central square (3.41 vs. 4.25) and run for a longer time (7.66 vs. 5.48 s) (Table 3.6). Females were less prone to an active exploration of a new environment: they showed higher latency to enter the arena (24.0 vs. 19.7 s) and stood still (57.7 vs. 45.6 s) for longer; whereas, they spent less time grooming (6.03 vs. 7.84 s) and biting (5.16 vs. 6.51 s) compared to males (Table 3.6).

Table 3.5

Response to the tonic immobility test at 66 and 73 d of age: effect of environmental enrichment, age, gender and their interactions

	Platform (P)		Tube (T)		Age (A)		Gender (G)		P-value						
	with	without	with	without	66 d	73 d	Males	Females	P	T	A	PxT	PxA	TxA	G
Rabbits (n)	80	80	80	80	80	80	81	79							
Sensitive rabbits (%)[a]	100	97.5	98.5	100	98.8	98.8	98.8	98.7	0.15	0.15	0.99	0.11	0.57	0.57	0.99
Attempts (n)	1.24	1.18	1.23	1.19	1.31	1.11	1.19	1.22	0.73	0.79	0.26	0.82	0.60	0.68	0.86
Immobility (s)	87.0	69.9	72.7	83.6	74.5	81.6	77.7	78.3	0.26	0.47	0.64	0.23	0.53	0.26	0.66

[a]Rabbits that exhibited immobility within three attempts

Table 3.6

Behaviours during the open field test at 65 and 72 d of age: effect of environmental enrichment, age, gender and their interactions

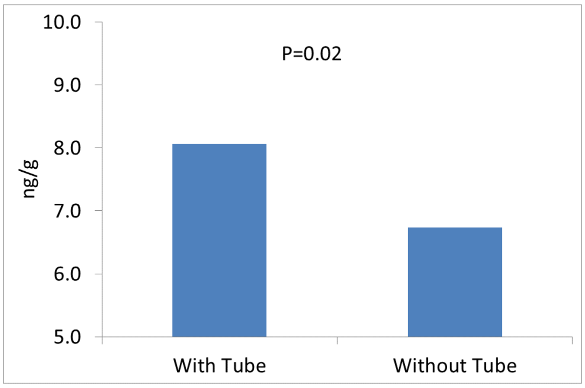
	Platform (P)		Tube (T)		Age (A)		Gender (G)		P-value						
	with	without	with	without	65 d	72 d	Males	Females	P	T	A	PxT	PxA	TxA	G
Rabbits (n)	80	80	80	80	80	80	80	80							
Entered animals[a,b] (%)	78.8	87.5	87.5	78.8	83.8	82.5	82.5	83.8	0.14	0.14	0.83	0.22	0.49	0.45	0.83
Latency (s)	23.9	15.5	22.5	21.0	21.1	22.4	19.7	24.0	0.28	0.69	0.72	0.67	0.58	0.67	<0.001
Total displacements (n)	52.3	49.7	49.6	52.4	48.2	53.9	51.1	50.8	0.42	0.40	0.09	0.29	0.91	0.91	0.79
Central displacements (n)	3.75	3.88	3.68	3.95	4.25	3.41	3.46	4.20	0.70	0.42	0.01	0.02	0.17	0.61	0.08
Exploration (s)	529	516	52.6	519	511	534	516	528	0.06	0.32	<0.01	0.19	0.72	0.65	<0.001
Movement (s)	39.2	39.2	38.5	39.9	38.0	40.4	38.3	40.0	0.99	0.56	0.28	0.03	0.72	0.26	0.09
Running (s)	7.24	5.79	5.74	7.30	5.48	7.66	6.85	6.12	0.13	0.10	0.02	0.94	0.88	0.26	0.07
Standing still (s)	50.8	51.8	49.0	53.7	46.9	56.1	45.6	57.7	0.86	0.39	0.09	0.42	0.56	0.47	<0.001
Grooming (s)	8.05	5.87	7.37	6.40	6.75	6.99	7.84	6.03	0.05	0.37	0.82	<0.01	0.07	0.84	<0.001
Biting (s)	6.21	5.42	5.01	6.71	2.87	11.7	6.51	5.16	0.72	0.46	<0.001	0.20	0.78	0.02	<0.001

[a] Rabbits that entered the pen spontaneously within 60 s

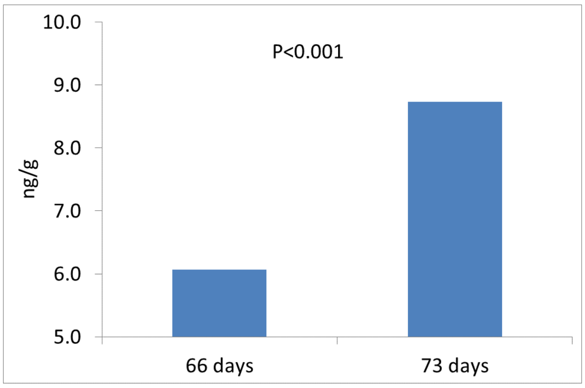
[b] Probability of χ^2 test

Corticosterone in hair

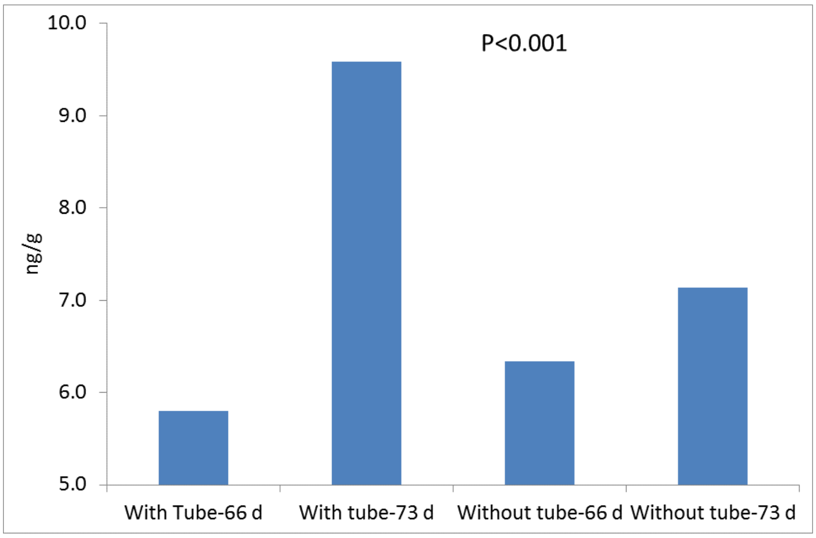
The presence of the platform had no effects, while the presence of the tube increased corticosterone concentration in hair (8.06 vs. 6.74 ng/g; P=0.02) (Figure 3.8). The increase of age from 66 to 73 d increased corticosterone concentration (6.07 to 8.73 ng/g; P<0.001) (Figure 3.8). A significant interaction was measured between the presence of the tube and the animal age (Figure 3.8): hair corticosterone concentration increased more in rabbits kept in pens with tubes (hair: 5.80 to 9.59 ng/g) than in pens without tubes (hair: 6.34 to 7.14 ng/g) (P<0.001).



(a)



(b)



(c)

Figure 3.8.Hair corticosterone concentration (ng/g) in the pens with and without tube (a), at 66 and 73 d of age (b) and as a result of the interaction between tube and age (c).

Discussion

Effect of the platform

In our conditions, the presence of the platform in the collective pens did not modify to a great extent growth performance. In accordance with EFSA, which established the 40 kg/m^2 at slaughter as a “safe” threshold for growing rabbits, at the end of the present trial 40.5 and 40.2 kg/m^2 were measured in pens with and without the platform respectively (the surface of the platform was included in the calculation of available surface) (EFSA, 2005). Therefore, we could argue that stocking density was sufficiently low to guarantee good general conditions of animals (and satisfying performance) regardless of platforms. As already found by other authors in rabbits kept collectively in pens without and with platform, the latter gives animals the opportunity to distribute themselves more freely into the pen providing them with an additional floor area without negatively affecting their growth performance (Matics et al., 2014; Szendrő et al., 2009a; Szendrő et al., 2012a).

However, at the end of the present trial, rabbits kept on platform showed a higher occurrence of injuries which may be explained by a higher level of aggressiveness among animals, even if it was not confirmed by the ethogram. The presence of the platform *per se* could not justify this result; it could rather have happened due to the higher group size in the pens with platforms compared to those without. At increasing group size, higher difficulties in establishing hierarchies may be expected, as already observed by Lambertini et al. (2005) and Princz et al. (2008a). In fact, wild rabbits establish their sex-specific social rank in rather restricted living groups (until 9 animals) (Schlolaut et al., 2013).

Despite the higher occurrence of injuries when platform was present, corticosterone levels were not affected which may be explained by differences in corticosterone level of rabbits according to their position in the hierarchies (and therefore in stress levels). Since also the ethogram did not evidence differences in the occurrence of aggressions, we could argue that probably only few (one, two?) animals within a pen attacked the other animals, which occurred evenly (even if produced injuries) to an important rate and therefore it was not evidenced at the behavioural observations.

Differently, the enrichment of the pens with platforms permitted to widen the behavioural pattern of rabbits, which were capable of hopping from and to the platforms and hiding below, as it was reported also by Combes et al. (2010) and Postollec et al. (2008). Additionally, rabbits in the presence of the platform showed a reduction of some behaviours like self-grooming and allo-grooming which may be considered as filling behaviours when performed at high rates. The meaning of the increase of resting with body in the stretched body position (rather than crouched) is more difficult to explain. In the first and second trials of the present thesis, rabbits used to assume the body crouched position more than the stretched, regardless of floor type, age or housing system. In the study of Jordan et al. (2011), when rabbits were reared individually in wire-net cages, they preferred to rest with their body crouched rather than stretched showing differences according to the time of day (rested more in the stretched position during afternoon and the first half of night).

The day-time rabbits stayed above platform especially during the hours of major activity (the dark hours) and under the platform during the hours of resting, probably using it as a refuge area. Similarly, Szendrő et al. (2012a) noticed a preference of rabbits for the area under the platform than that above.

Rabbits dedicate most of their time resting in both a wild environment (with variations depending on the vegetation structure and food availability) (Kolb, 1986; Lombardi et al., 2007; Selzer and Hoy, 2003) and in farm conditions (Princz et al., 2008a; Trocino et al., 2013; present trial). Under this latter conditions, main activities are resting, self-grooming, and feeding regardless of the housing system used or rabbit age (Buijs et al., 2011b; Dal Bosco et al., 2002; Trocino et al., 2008, 2013).

Effects of the tube

The use of a plastic tube as environmental enrichment was chosen as an element to stimulate rabbits to perform behaviours other than social, like hiding, resting and phisical activities. It was expected that this intervention would have been resulted in reducing animals stress created by the general rearing practices and conditions and that would have been ammeliorated relationships among rabbits diminishing their aggressiveness without impairing productivity. Instead, growth performance was negatively affected, whereas reactivity towards human or a new environment had no advantage from the presence of the tube and rabbits behaviour during 24-hours did not show any improvement; conversely, rabbits did not use the tube that much. The latter could have probably happened because of the unsuitability of the tube; maybe the diameter was too small, especially for the older rabbits, not favoring their passage or creating an unfriendly micro-environment for them. Also, introducing the plastic tube into the pens worsened stress level measured by corticosterone concentrations.

Studies that have examined the effects of tubes or hiding structures in rabbits are scarse. Tuyttens et al. (2005) did not report important effects on growth performance when different elements (platform, wooden stracture for hiding, wooden sticks) were simultaneously presented in the cage of rabbits under intensive rearing systems. Hansen and Berthelsen (2000) enriched the cages of individually reared laboratory rabbits with a box in which animals could hide themselves in and they noticed that only few rabbits, and especially females, used the box as a refuge or resting area; they rather used its roof for sighting or resting. Similarly, Lindfors (1997) verified that when rabbits had the opportunity to use a box for hiding they rarely used it and performed the same abnormal behaviours of the animals without environmental enrichment in their cages; whereas growth performance was similar between these two groups.

Literature concerning enrichment elements for rabbits, other than tubes or hiding boxes, is extensive and evaluated metallic or wooden objects placed on the cage or in suspension by the ceiling (Maertens and Van Oeckel, 2001; Mirabito et al., 2000; Verga et al., 2004) or, even, mirrored surfaces (Dalle Zotte et al., 2009a; Edgar and Seaman, 2010; Jones and Phillips, 2005).

Effect of age

Generally speaking, the results obtained with the increase of the age from 68 to 75 days of age confirm previous studies conducted on rabbits reared in single or bicellular cages (Cavani et al., 2009; Dalle Zotte et al., 2000; Parigi Bini et al., 1992). In case of group rearing, the worsening of growth performance may be more pronounced than the one observed under conventional housing because of a simultaneous increase of aggression and competition among animals. Szendrő et al. (2009b) noticed that the number of lesions on rabbits ears was low at 9 weeks of age, but it was tripled until the 11th week. Similar results were obtained by Rommers and Meijerhof (1998). Actually, it seems that rearing in large groups seems to stimulate sexual maturity of rabbits, which manifest sexual behaviours earlier compared to animals reared in single and bicellular cages or in small groups at the same age and at higher live weight. This was confirmed by the present trial, as the percentage of animals with lesions on genitals augmented with slaughter age.

Few behavioural measurements change with increasing age, feeding time decreases and grooming increases (Martrenchar et al., 2001; Morisse et al., 1999; Trocino et al., 2013; present trial); there is also a higher frequency of running and hopping (Trocino et al., 2013; present trial), which may be associated with the appearance of sexual and aggressive interactions among animals.

Reactivity towards a new environmental situation changed: on the one hand, they increased cautious exploratory activities, but on the other hand they were more tense running less and reducing their passage from the centre of the arena, as already found in the Trial No. 2 of the present Thesis.

The higher levels of corticosterone measured in the older rabbits, likely due to sexual maturity, was confirmed also by the first experiment of the present thesis.

Effect of gender

The housing of growing rabbits in bicellular cages, the early age of slaughter and the weak sexual dimorphism are the reasons for which rabbits, under intensive rearing, are reared in mixed-gender groups. Nevertheless, housing in single-gender groups would be wise when alternative housing will be applied because of the increase of competition and "sexual" interactions.

In the present trial, the percentage of rabbits with lesions on their body was higher in males than females and of higher severity. The difference attributed to the higher percentage of injuries that males appeared on genitals compared to females confirming the results obtained in the second trial of the thesis. Though, it is not clear if these injuries resulted by the aggressions of the females towards the males for defending themselves or by the occurrence of struggles among males approaching sexual maturity.

Rabbits reactivity towards human was not influenced by gender; whereas, towards a new environment females resulted more afraid being less prone to enter the arena, to perform self-grooming and bite elements of the arena and spent more time standing still compared to males. In the Trial No. 2 of the present Thesis, gender also influenced reactivity of rabbits towards human and a new environment but in a different way compared to the present trial: males were more bold during the tonic immobility test (stayed for less time immobile) but more afraid during the open field test (less locomotory and comfortable behaviours into the arena). To our knowledge, studies comparing the reactivity of intensively reared rabbits between the two genders are limited.

Conclusions

The presence of the platform did not alter growth performance. Anyhow, at the end of the trial and before slaughter the percentage of rabbits with genital injuries was higher in the pens equipped with platform than those without, even if the behavioural observations did not reveal any difference in aggressive manifestations. The presence of a platform allowed the enrichment of rabbits behavioural repertoire, which had the opportunity to perform more activitites (i.e. jump, hide).

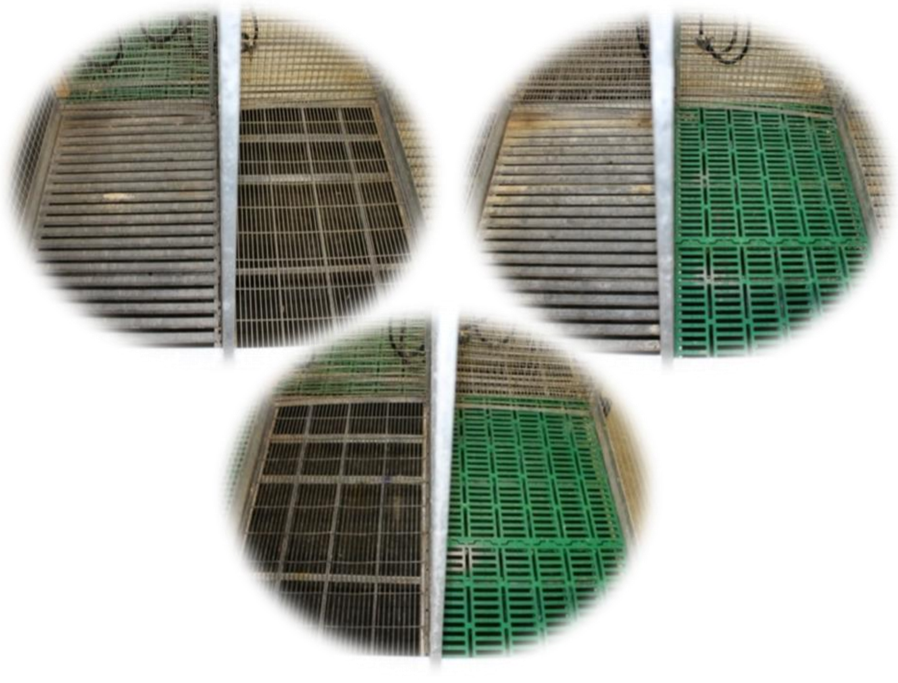
Concerning the use of the plastic tube, growth performance and stress level were impaired when the tube was used. Also, the percentage of rabbits with body injuries was augmented and no advantage in behaviour was noticed. At this point it could be said that the use of this type of tube, as the one tested in this trial, is not recommended; maybe changing the characteristic of the tube (more wide or with openings on the surface, etc.) different results would have been obtained.

Regarding the effect of slaughter age and gender, an increase of genitals lesions with the increase of age and in the males than females was confirmed.

To sum up, rearing in pens equipped with platform allows the increase of the number of rabbits housed irrespective of the available space per animal without negative effects, whereas the tube impairs productivity and stress of animals. With the increase of slaughter age negative effects become more intense, which is accompanied by an increase of the signs of aggressiveness among rabbits. At last, after clarifying some points still uncertain, rearing separately by gender in large groups as a way to diminish the incidence of injuries would be a new possibility of alternative pen housing for growing rabbits.

Trial No.4:

Effect of different gender composition and floor combinations on welfare and productivity of growing rabbits housed in collective pens



Introduction

Survey conducted at European level emphasized that more and more European citizens (the 82% of the 25,000 consumers tested) urge for techniques that respect animal welfare during rearing (Eurobarometer, 2007). Focusing on growing rabbits sector, this means renovate current housing systems (small groups: 2-8 rabbits/pen; 14-23 animals/m^2; wire-net floor) (Trocino and Xiccato, 2006) and management (i.e. feeding strategy, sex composition), so as a balance among society demand, producers profit and rabbit welfare will be achieved.

Searching for alternative methods, rearing rabbits in pens and in large groups (>10 animals/pen) seems to have positive effects, as the animals may demonstrate behaviours other than resting or feeding (Princz et al., 2008a); however, attention should be paid when group size exceeds 20 animals per pen, as lesions due to aggressions may increase (Szendrő et al., 2009b). At the same time, with the increase of group size, growth performance, slaughter and meat quality traits may get worse (Lambertini et al., 2001). In fact, in the Trial No. 1 of the present Thesis rabbits reared in large groups (>20 animals/pen) exhibited more species-specific behaviours and no stereotypies compared with those reared collectively. Though, feed consumption, growth rates and dressing percentages were worse in rabbits reared collectively.

To date, group housing of growing rabbits takes place in mixed-gender groups (females and males together), as sexual dimorphism is low and they are led to slaughter before reaching sexual maturity (until about 80-85 d of age). Studies have shown few differences in growth performance and meat quality characteristics among sexes (Di Meo et al., 2003; Lazzaroni et al., 2009; Trocino et al., 2003; Trial No. 3); whereas, approaching sexual maturity, aggressions to settle hierarchies may be frequent (Verga et al., 2006) rising the stress and fear levels. Both in the Trial No. 2 and 3 of the present Thesis, males had a higher percentage of injuries compared with females; while in the Trial No. 2 the occurrence of injuries was augmented as rabbits were getting older.

Floor type is another critical point both from the animal (as rabbits spend all of their time on it) and the farmer side (because of its costs and its role in maintaining the hygiene of the farm). Materials other than the current wire-net have been tested in various studies. Deep litter is believed to worsen sanitary status as it favours the accumulation of animal waste and therefore diseases transmission (EFSA, 2005), besides being less preferred by rabbits and it is usually correlated with poor productivity traits (Morisse et al., 1999; Orova et al., 2004; Trocino et al., 2008). Contrary, rabbits seem to have high preference towards plastic-net (Princz et al., 2008a). An inappropriate floor may impair growth, stress and fear level. Indeed, the use of wooden slat floor with distance between slats of 3 cm, in the Trial No. 1 and 2 of the present Thesis, proved the latter statement.

The present study aimed at comparing behaviour, reactivity and stress level of growing rabbits reared in groups (16 animals/pen) of the same or mixed gender (only males, females or both), in open-topped pens composed of two freely-communicating modules with different floor combinations (steel/plastic vs. steel/wire-net vs. plastic/wire-net).

Materials and Methods

Animals and housing

This study was approved by the Ethical Committee for Animal Experimentation of the University of Padova and funded by the University of Padova. All animals were handled according to the principles stated in EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

The trial was held in the experimental stable of the University of Padova with natural photoperiod (11-13 h daylight), equipped with a forced heating and cooling system to achieve temperature ranging from 18 and 21^{o}C.

From the day after weaning (33 d of age), a total of 288 Hyplus crossbred rabbits housed in groups (16 rabbits/pen) in 18 open-top pens were controlled until slaughter (75 d of age). To their arrival, the animals were ear-tagged and individually registered by gender. The pens were consisted of two modules (each of them: 64 cm-wide, 78 cm-long, 110 cm-high, usable area: 0.5 m^2/module) and at stocking density equal to 16 animals/m^2, communicating to each other by a centrally located opening (23 cm-wide and 25-cm high) on the common wire-net wall, which permitted the animals free passage along the two modules (Figure 4.1). Each of the modules was equipped with different floor types; galvanised wire-net, steel slat or plastic slat. Hence, the galvanised wire-net had hole dimensions: 10 mm-wide, 70 mm-long and diameter: 3 mm; the steel slatted floor had quadratic slats put transversely to the pens with 20 mm-section and distance between the slats equal to 15 mm; and the plastic slatted floor had slats: 70 mm-long, 10 mm-wide and distance between slats equal to 7 mm (Figure 4.2).

(a) (b)

Figure 4.1. Open-top collective pen consisted of two modules (a) connected to eachother by a hole (b).

So, three floor combinations of pens were obtained: steel slats/wire-net vs. steel slats/plastic slats vs. wire-net/plastic slats, which were repeated three times to include the three groups of gender: only females vs. only males vs. half females + half males.

(a) (b) (c)

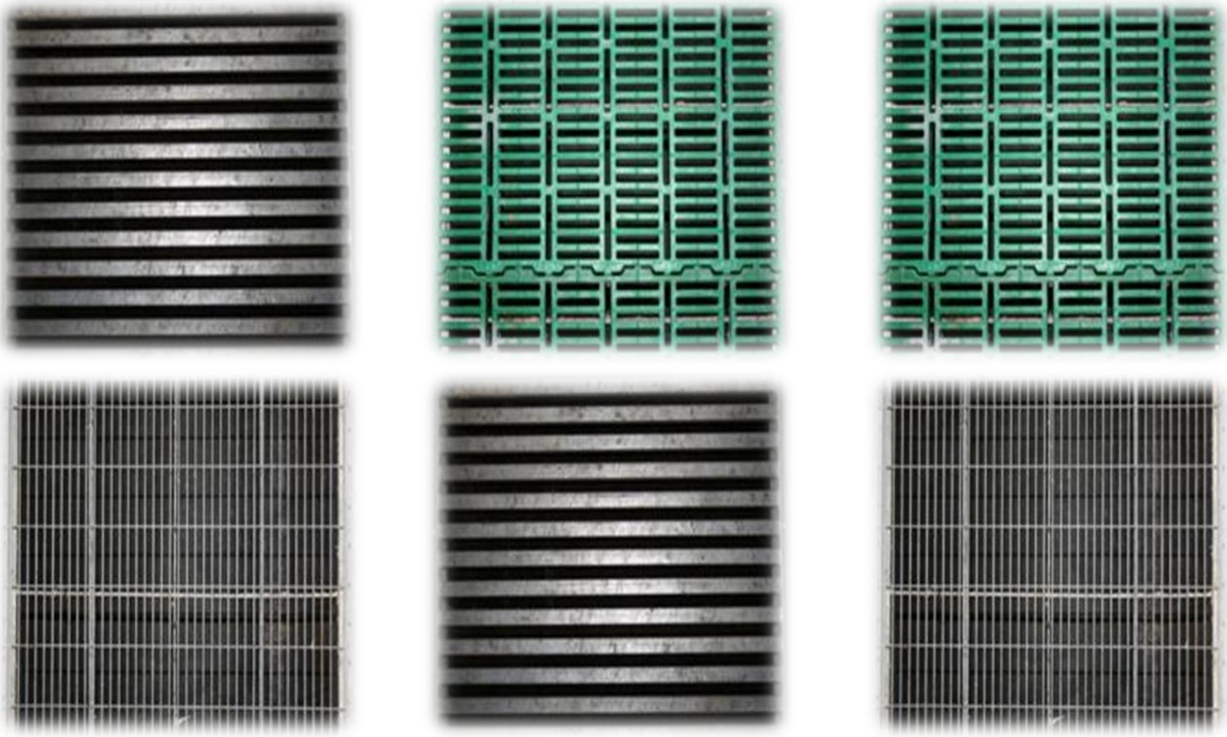


Figure 4.2. Floor combinations: steel slats/wire-net (a), steel slats/plastic slats (b), wire-net/plastic slats (c).

Animals had free access to nipple drinkers (two per module) and free access to manual feeders (two per module). The feeders (43.5 × 11.5 × 30 cm) were placed on the front wire-net wall, whereas on the back wall the drinkers were placed (43-cm distance between them). Two pelleted commercial diets were given during the trial: a post-weaning diet with coccidiostatic (provided from weaning until 54 d of age) and a not medicated and coccidiostatic-free growing diet (provided from 55 d of age until slaughter). The diets were formulated to satisfy rabbits requirements according to De Blas and Mateos (2010): 89.0% and 87.6% of dry matter, 15.8% and 15.1% of crude protein, 35.4% and 34.8% of NDF, 19.7% and 19.0% of ADF, 4.8% and 4.6% of ADL, for the post-weaning and growing diet, respectively.

During the experiment, the animals maintained a satisfied health status and only 4 rabbits died.

Recordings

Rabbit individual weight was controlled twice per week and pen feed consumption daily. Everyday monitoring of the animals took place to ensure they were free of any clinical sign of disease.

Behavioural budget-time was evaluated by applying 24-hours video-registrations (2 min per hour) on all the animals (288 rabbits) at 42-43 d (initial period), 56-57 d (interim period) and 69-70 d (final period) of age by using a digital video-recorder (VRD160CD, RDS CCTV Srl, Montesilvano, PE, Italy). The analysis of behaviours was performed as described in the Trial No. 1 and 3.

Rabbits floor preference was evaluated once per week, three times per day (at 8.00, 13.00 and 18.00 h) by video-recording their instant distribution in each of the two modules of every pen.

The reactivty tests were performed and analysed as described in the Trial No. 1 and 2.

The open-field test was realized on a total of 108 rabbits (6 animals × 18 pens), at 66 d of age. The selection of the rabbits took place following the same criteria with the tonic immobility test. The animals undergone this test were not submitted to the tonic immobility.

The tonic immobility test was performed on 108 rabbits (6 animals × 18 pens) at 67 d of age by testing two animals per pen and repeating this sequence three times using different animals (three rounds/pen). In the mixed pens (both males and females), the sequence of the test was realised by using one male and one female every time.

At 68 d of age, the novel object test was performed on all the pens using a bottle. The human contact test was performed at 69 d of age, on all the pens.

At 65 d of age, hard faeces and hair were sampled for corticosterone determination in 96 rabbits (singled-gender pens: 5 animals × pen; mixed-gender pen: 3 females and 3 males × pen). The corticosterone levels were measured as described in the Trial No 1.

Commercial slaughter

At 75 d of age, 275 animals (4 animals died and 9 animals were excluded because their live weight was <2.2 kg) were individually processed in a commercial slaughterhouse and the slaughter took place following the same procedures of the Trial No. 1.

Statistical analysis

Individual data were submitted to ANOVA using the PROC MIXED of SAS (Statistical Analysis System Institute, 2013). Floor type/combination and gender composition were the principal factors and pen the random effect. The pen data for feed intake and feed conversion were analysed using the PROC GLM with floor type and gender composition as principal factors. The behavioural, reactivity and stress data were submitted to mixed procedures as those used and described in the previous trials.

The behavioural, reactivity and stress data were tested for normality. The normally distributed data were analyzed by using the PROC MIXED with floor type and gender composition as main effects and pen as random effect. The behavioural variables not normally distributed were analysed through a non-parametric analysis (Kruskal-Wallis and Mann-Whitney tests) using the PROCNPAR1WAY with floor type and gender composition as main effects.

Results

Animals final live weight, daily weight gain and feed intake were not affected by any of the experimental factors (Table 4.1).

Animals distribution on the modules: preference for the floor type

Notable was the rabbits preference for the different types of floor represented by the distribution of animals on the various modules. On average 5.57 rabbits were observed to occupy the steel slatted modules, 7.79 rabbits the wire-netted modules and 10.64 rabbits the plastic slatted modules (data not provided in the table, P<0.001).

The graphic depiction of the number of rabbits present on the two modules of each experimental group (average of observations at different ages) confirms the differences of animals distribution according to the type of floor within group (the effect of floor type × experimental group was significant at level of P<0.001) (Figure 4.3).

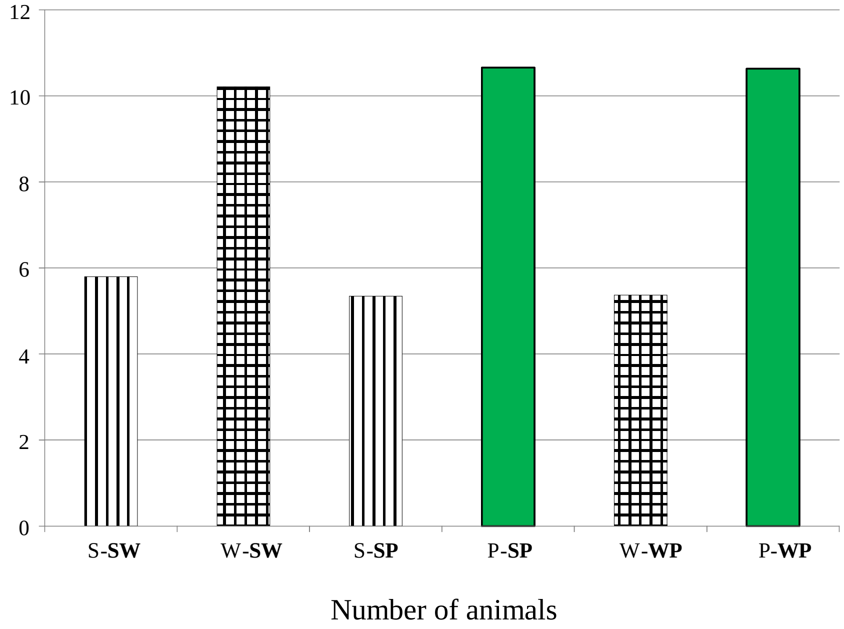


Figure 4.3. Number of animals present in the modules of different floor type (S: steel slat; W: wire-net; P: plastic slat) and according to the different experimental groups (SW: steel/wire-net; SP: steel/plastic; WP: wire-net/plastic): average of the observations at different ages. Effect of floor type × experimental group (P<0.001).

Table 4.1

Rabbits performance from 33 d of age (weaning) to slaughter: effect of floor combination, gender composition and their interaction

	Floor type (F)			Gender composition (G)			P-value			RSD
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G	F×G	
Pens (n)	6	6	6	6	6	6				
Final live weight (g)										
at 33 d of age	830	831	833	830	831	833	0.37	0.28	0.27	3.5
At 74 d of age	2865	2874	2840	2881	2824	2873	0.78	0.51	0.71	84.8
Daily weight gain (g/d)										
From 33 d to slaughter	49.6	49.8	48.9	50.0	48.6	49.7	0.75	0.51	0.71	2.1
Daily feed intake(g/d)										
From 33 d to slaughter	149	149	145	148	147	147	0.56	0.95	0.83	6.7
Conversion index										
From 33 d to slaughter	3.00	2.99	2.96	2.96	3.03	2.96	0.42	0.09	0.29	0.04
Dressing percentage (%)	61.1	60.7	61.2	60.2	61.8	61.0	0.21	<0.001	0.22	1.73

Besides, it shows a lower number of animals on the modules equipped with steel slat floor; both when the second modules had wire-net (SW cages) or plastic slat floor (SP cages). On the other hand, the highest number of animals was observed on the modules with plastic floor, both when steel slat (SP cages) and wire-net (WP cages) floor were present as the second module.

When the average distribution on the modules with the three types of floor is presented according to the age of the animals, the preference of the younger animals for the plastic floor is clear (Figure 4.4). Nevertheless, from 59 days of age, the distribution of the animals on the modules with the three floor types was similar (the effect of floor type × age was significant at level of P<0.001).

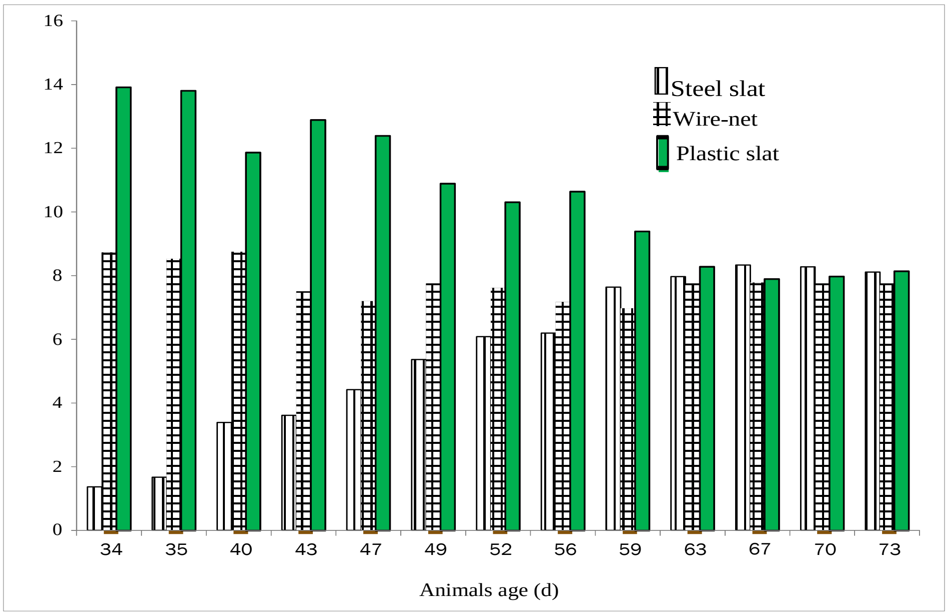


Figure 4.4. Number of animals presented in the modules of different floor types (S: steel slat; W: wire-net; P: plastic slat) at different ages (effect of floor type × age, P<0.001).

Behaviour during the 24-h period

The evaluation of animals behaviour at 42, 56 and 69 d of age (Tables 4.2, 4.3, 4.4) showed that, on average, the main activities were: resting (62.4, 61.3 and 67.4% of the time observed at the three ages, respectively), feeding (13.1, 11.4 and 8.8%), self-grooming (10.9, 16.3 and 15.7%), allo-grooming (2.45, 1.86 and 1.29%), drinking (2.00, 2.01 and 1.95%), moving (1.78, 1.28 and 1.04%) and explorative behaviours, like sniffing (1.97, 1.46 and 1.15%) and licking pen elements (2.54, 3.83 and 1.25%).

Table 4.2

Behaviours during video-recording at 42-43 d of age (% of budget time or number of events): effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Observations (n)	96	96	96	96	96	96		
Preference 2nd module[a] (%)	68.8	80.5	74.6	77.5	74.8	71.7	<0.01	0.03
Feeding (%)	13.1	13.1	13.0	12.6	13.5	13.1	0.95	0.78
Drinking (%)	1.85	1.98	2.17	2.02	2.15	1.85	0.60	0.61
Moving (%)	1.81	1.85	1.66	1.90	1.58	1.84	0.22	0.04
Running (%)	0.36	0.34	0.39	0.42	0.33	0.34	0.37	0.21
Hopping (%)	0.03	0.03	0.07	0.03	0.02	0.07	0.43	0.15
Self-grooming (%)	12.0	10.2	10.5	10.6	11.5	10.5	0.04	0.35
Allo-grooming (%)	2.62	2.35	2.32	2.48	2.63	2.17	0.47	0.36
Total resting (%)	61.2	63.5	62.5	62.5	61.3	63.5	0.32	0.51
Body crouched (%)	51.2	46.3	45.5	46.8	48.4	47.9	<0.01	0.60
Body stretched (%)	10.0	17.2	17.0	15.7	12.9	15.5	<0.01	0.10
Rearing (%)	0.14	0.19	0.24	0.23	0.15	0.18	0.27	0.62
Sniffing (%)	2.11	2.03	1.75	2.23	1.97	1.70	0.11	0.20
Licking cage (%)	2.38	2.59	2.64	2.65	2.53	2.43	0.71	0.72
Caecotrophy (%)	0.69	0.36	0.57	0.50	0.64	0.49	0.02	0.81
Sitting (%)	1.59	1.46	2.09	1.71	1.67	1.76	<0.01	0.72
Aggressions (%)	0.05	0.006	0.05	0.04	0.04	0.02	0.15	0.21
Stretching (%)	0.03	0.02	0.04	0.02	0.03	0.04	0.10	0.50
Hops (n)	0.81	0.77	0.72	1.31	1.01	1.04	0.20	0.05
Alerts (n)	1.01	1.08	1.27	1.02	0.61	0.67	0.67	0.79
Aggressions (n)	0.03	0.01	0.10	0.02	0.02	0.10	0.13	0.18

[a]Percentage of rabbits presented in the 2nd module (i.e. in the module with wire-net floor in the pens with steel/wire-net floor)

Table 4.3

Behaviours during video-recording at 56-57 d of age (% of budget time or number of events): effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Observations (n)	96	96	96	96	96	96		
Preference 2nd module[a] (%)	55.1	63.8	64.7	65.9	60.9	56.8	<0.01	0.01
Feeding (%)	10.7	11.3	12.0	11.8	10.6	11.5	0.21	0.27
Drinking (%)	1.97	2.12	1.95	2.09	1.89	2.06	0.58	0.67
Moving (%)	1.51	1.27	1.05	1.11	1.40	1.33	<0.01	<0.01
Running (%)	0.18	0.22	0.18	0.14	0.23	0.21	0.57	0.04
Hopping (%)	0.005	0.007	0.002	0.006	0.004	0.004	0.05	0.66
Self-grooming (%)	16.3	16.7	16.0	16.3	16.4	16.2	0.73	0.90
Allo-grooming (%)	1.72	1.98	1.89	1.49	2.08	2.02	0.58	0.02
Total resting (%)	61.1	61.2	61.6	62.1	60.9	60.9	0.92	0.84
Body crouched (%)	48.2	43.9	46.4	45.6	47.1	45.7	0.02	0.50
Body stretched (%)	12.9	17.3	15.3	16.4	13.8	15.2	<0.01	0.06
Rearing (%)	0.09	0.09	0.07	0.10	0.06	0.08	0.09	0.34
Sniffing (%)	1.71	1.46	1.20	1.03	1.91	1.44	0.02	<0.01
Licking cage (%)	2.83	2.32	2.53	2.35	2.81	2.52	0.23	0.24
Caecotrophy (%)	0.71	0.50	0.79	0.69	0.66	0.65	0.35	0.69
Sitting (%)	1.04	0.84	0.69	0.68	0.97	0.93	0.05	0.02
Aggressions (%)	0.11	0.07	0.02	0.03	0.10	0.06	0.34	0.76
Stretching (%)	0.027	0.013	0.011	0.02	0.009	0.02	0.54	0.55
Digging (%)	0.000	0.020	0.000	0.004	0.000	0.013	0.13	0.61
Hops (n)	0.14	0.19	0.06	0.49	0.34	0.31	0.04	0.67
Alerts (n)	0.42	0.43	0.30	0.15	0.14	0.10	0.09	0.20
Aggressions (n)	3.20	2.14	0.50	1.01	3.00	1.82	0.39	0.78

[a]Percentage of rabbits presented in the 2nd module (i.e. in the module with wire-net floor in the pens with steel/wire-net floor)

Table 4.4

Behaviours during video-recording at 69-70 d of age (% of budget time or number of events): effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Observations (n)	96	96	94	96	96	94		
Preference 2nd module[a] (%)	48.3	48.2	50.5	49.8	48.9	48.2	0.82	0.72
Feeding (%)	8.90	8.73	8.77	8.95	8.92	8.53	0.92	0.81
Drinking (%)	2.19	1.87	1.78	1.98	1.82	2.04	0.43	0.96
Moving (%)	1.19	0.83	1.09	0.50	1.66	0.95	0.31	<0.001
Running (%)	0.30	0.27	0.34	0.16	0.48	0.27	0.20	<0.001
Hopping (%)	0.005	0.005	0.005	0.006	0.007	0.003	0.90	0.39
Self-grooming (%)	15.3	16.1	16.3	18.4	15.4	13.9	0.45	<0.001
Allo-grooming (%)	1.00	1.85	1.01	0.88	1.82	1.17	0.02	<0.001
Total resting (%)	67.0	67.4	67.7	66.5	65.9	69.7	0.94	0.01
Body crouched (%)	46.6	45.5	46.3	40.0	49.7	48.6	0.68	<0.001
Body stretched (%)	20.4	21.9	21.4	26.5	16.2	21.0	0.56	<0.001
Rearing (%)	0.03	0.07	0.03	0.05	0.05	0.04	0.31	0.96
Sniffing (%)	1.49	0.89	1.06	0.53	1.62	1.30	0.07	<0.001
Licking cage (%)	1.49	1.24	1.00	1.29	1.26	1.18	0.14	0.41
Caecotrophy (%)	0.52	0.36	0.31	0.45	0.42	0.31	0.37	0.67
Sitting (%)	0.40	0.37	0.58	0.29	0.49	0.57	0.56	<0.01
Aggressions (%)	0.05	0.02	0.05	0.02	0.05	0.04	0.55	0.23
Stretching (%)	0.14	0.03	0.03	0.02	0.14	0.03	0.11	<0.01
Digging (%)	0.14	0.03	0.03	0.02	0.14	0.03	0.11	<0.01
Hops (n)	0.13	0.13	0.12	0.19	0.26	0.18	0.90	0.38
Alerts (n)	0.17	0.29	0.18	0.15	0.15	0.07	0.50	0.99
Aggressions (n)	0.07	0.04	0.04	0.02	0.07	0.06	0.54	0.23

[a]Percentage of rabbits presented in the 2nd module (i.e. in the module with wire-net floor in the pens with steel/wire-net floor)

Floor type significantly affected the budget time at the three ages. More specifically, at 42 d of age, the effect of the floor type on behaviour confirmed the results of the preference test above mentioned (Table 4.2): in the pens with steel/wire-net floor, the 68.8% of the rabbits preferred the wire-netted module; in the pens with steel/plastic or wire-net/plastic floor, an even larger proportion of the rabbits (80.5 and 74.6%, respectively) preferred to stay on the plastic floor module.

Some minor differences were noticed among groups: in the steel/wire-net pens the time spent self-grooming was higher (12.0 vs. 10.2 and 10.5%, P=0.04) compared to the modules they were containing plastic floor. Time spent resting (totally) was the same among the three types of pens; but, in the steel/wire-netted pens rabbits adopted for more time the crouched position during resting (51.2% in steel/wire net pens, 46.3% in steel/plastic pens, 45.5% of the observed time in wire net/plastic pens; P<0.01) and for less time the stretched position (10.0, 17.2, 17.0%; P<0.01). The same group of rabbits spent more time practicing caecotrophy compared to the others (0.69, 0.36, 0.57%; P=0.02). On the other hand, rabbits reared on the wire/plastic modules spent more time sitting than the others (2.09, 1.59, 1.46%; P<0.01).

At 56 d of age, time spent on the different modules was, again, different depending on the floor type (Table 4.3). The distribution tended to be more homogeneous between the two modules of the steel/wire net pens, but not when the pens were equipped with plastic floor (steel/plastic or wire/plastic pens). In the latter case, time spent in the module with the plastic floor was notably higher than the eventual second module. In the pens with steel/wire-net floor, rabbits spent more time moving (1.51% in steel/wire net pens, 1.27% in steel/plastic pens, 1.05% wire net/plastic pens; P<0.01), sniffing (1.71, 1.46, 1.20%; P=0.02) and sitting (1.04, 0.84, 0.69%; P=0.05). The same rabbits rested less with their body stretched (12.9, 17.3, 15.3%; P<0.01) and more with their body crouched (48.2, 43.9, 46.4%; P=0.02) compared with the rabbits reared in the pens containing plastic floor.

At 69 d of age, rabbits distribution in the modules of a pen was the same (48-52%) and budget time was more or less the same among modules (Table 4.4). Only the time spent allo-grooming was higher in the rabbits of the steel/plastic pens (1.85%) compared with the rabbits of the steel/wire-net (1.00%) and wire-net/plastic pens (1.01%) (P=0.02).

Gender composition within pens also affected rabbits preference at 42 d of age (Table 4.2). Females demonstrated a clear preference for staying more on one module (77.5%), tending to remain grouped together compared to males (74.8%) and mixed groups (71.7%) (P=0.03). Less strong was the effect of the gender composition on the budget time during the first ethogram. The number of hops resulted significantly higher (P=0.05) in the female pens (1.31) compared to male

(1.01) or mixed-gender pens (1.04). Though, in the male pens time spent moving (1.58 vs. 1.90 and 1.84%; P=0.04) was lower compared to the other groups.

At 56 d of age (Table 4.3), the female tendency to stay on one of the two modules compared to male and mixed groups (65.9, 60.9, 56.8%; P=0.01, respectively), was again noticed. Time spent by male group resting with body stretched was maintained lower compared to the other groups (13.8, 16.4, 15.2%; P=0.06). Female group spent less time moving (1.11, 1.40, 1.33%; P<0.01), running (0.14, 0.23, 0.21%; P=0.04), allo-grooming (1.49, 2.08, 2.02%; P=0.02), sniffing (1.03, 1.91, 1.44%; P<0.01) and sitting (0.68, 0.97, 0.93%; P=0.02) compared to male and mixed groups.

At 69 d of age, the effect of gender composition on budget time was augmented (Table 4.4). In the female pens, less time devoted moving (0.50, 1.66, 0.95%; P<0.001), running (0.16, 0.48, 0.27%; P<0.001), sniffing (0.53, 1.62, 1.30%; P<0.001), allo-grooming (0.88, 1.82, 1.17%; P<0.001), sitting (0.29, 0.49, 0.57%; P<0.01), stretching and digging (0.02, 0.14, 0.03%; P<0.01); but, more resting with body stretched (26.5, 16.2, 21.0%; P<0.001) compared to male and mixed groups.

Reactivity towards man

Rabbits reactivity during the tonic immobility test was not affected by any of the experimental factors (Table 4.5) and all of the animals (100%) entered into immobility within three attempts.

Rabbits response to the human contact test was the same among the experimental groups (Table 4.6). On average, the 22% of rabbits contacted the operator within the first minute; whereas after five minutes, less than the half (46%) contacted, at least, once the operator.

Table 4.5

Response to the tonic immobility test at 67 d of age: effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Rabbits (n)	36	36	36	36	36	36		
Sensitive rabbits (%)[a]	100	100	100	100	100	100		
Attempts (n)	1.44	1.33	1.39	1.44	1.36	1.36	0.65	0.85
Immobility (s)	78.1	63.7	84.9	70.1	70.9	85.6	0.59	0.69

[a]The percentage of rabbits turned immobile within three attempts

Table 4.6

Response to the human contact test at 69 d of age: effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Rabbits (n)	96	96	94	96	96	94		
Rabbits (% of presented in the module) in contact with the operator within								
1st min	18.8	22.9	24.5	14.6	24.0	27.7	0.62	0.08
3rd min	35.4	37.5	39.4	33.3	39.6	39.4	0.85	0.60
5th min	42.7	47.9	47.9	44.8	51.0	42.6	0.71	0.48

Reactivity towards an unknown environment/stimulus

Floor type affected rabbits behaviour during the open-field test (Table 4.7). In details, the percentage of the rabbits entered spontaneously into the arena was higher in the pens with wire/plastic floor compared with the rabbits of the steel/wire and steel/plastic pens (64.7, 30.6, 33.3%; P=0.01, respectively). Additionally, the same group of rabbits (wire/plastic) realized a higher number of total (42.4, 30.4, 30.1; P=0.03) and central (3.56, 2.14, 1.86; P=0.09) displacements, spent more time running (3.59, 1.72, 1.78 s; P=0.03) and realized a higher number of hops (0.47, 0.19, 0.25; P=0.05) compared to the other groups.

Gender composition of groups did not affect the percentage of rabbits entered spontaneously into the arena; however differences in behaviour were noticed. Hence, rabbits of the mixed groups moved less (total displacing: 26.8, 38.2, 37.7; P=0.06 and moving: 30.6, 44.9, 36.7 s; P=0.05) and stood still longer (59.9, 45.9, 34.9 s, P=0.02) compared to the female or male group. The number of hops (0.50, 0.25, 0.17; P=0.04) and alerts (1.21, 0.81, 0.14; P=0.02) were higher in the rabbits reared in groups of males than females or mixed groups.

During the novel-object test, no effect of the experimental groups on rabbits percentage contacting the object was noticed (Table 4.8). Only the number of contacts (rabbits-object) differed depending on the gender composition, with male groups being more courageous compared to female and mixed groups (number of contacts: 8.15, 6.97, 6.51; P=0.01).

Corticosterone in hard faeces and hair

Hair corticosterone level was significantly affected by gender composition (Table 4.9), with rabbits of the mixed group having higher levels than female and male groups (6.65, 5.23, 5.87 ng/g; P=0.02).

Discussion

Effect of floor type: steel/wire-net vs. steel/plastic vs. wire-net/plastic

In the present trial, productivity traits were not influenced by floor type.Wire-net does not seem to compromise productivity traits of growing rabbits and it has been proved to promote higher hygiene levels and, consequently, welfare.

Table 4.7

Behaviours during the open-field test at 66 d of age: effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Rabbits (n)	36	36	36	36	36	36		
Entered animals[a] (%)	30.6	33.3	64.7	41.7	50.0	36.1	0.01	0.50
Attempts (n)	0.64	0.58	0.46	0.73	0.29	0.62	0.69	0.48
Latency (s)	27.9	33.3	29.5	30.2	27.2	33.7	0.39	0.29
Total displacements (n)	30.4	30.1	42.4	38.2	37.7	26.8	0.03	0.06
Central displacements (n)	2.14	1.86	3.56	2.81	2.79	1.92	0.09	0.29
Exploration (s)	373	366	369	369	380	359	0.87	0.18
Movement (s)	37.3	32.6	42.7	44.9	36.7	30.6	0.12	0.05
Running (s)	1.72	1.78	3.59	1.47	4.21	1.44	0.03	0.07
Stay still (s)	47.5	52.3	41.3	45.9	34.9	59.9	0.61	0.02
Grooming (s)	3.14	2.67	3.62	2.56	3.71	3.17	0.54	0.39
Biting (s)	15.8	20.3	20.0	16.5	18.7	20.9	0.90	0.92
Resting (lying) (s)	14.3	36.4	21.1	2.72	36.1	33.9	0.67	0.50
Digging (s)	0.14	0.75	0.09	0.00	0.15	0.83	0.77	0.17
Digs (n)	0.06	0.08	0.03	0.00	0.06	0.11	0.80	0.18
Hops (n)	0.19	0.25	0.47	0.25	0.50	0.17	0.05	0.04
Alerts (n)	1.08	0.31	0.74	0.81	1.21	0.14	0.36	0.02
Urinations (n)	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.99

[a]The percentage of rabbits entered, spontaneously, into the arena

Table 4.8

Response to the novel object test at 68 d of age: effect of floor combination and gender composition

	Floor type (F)			Gender composition (G)			P-value	
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G
Rabbits (n)	96	96	94	96	96	94		
Rabbits (% of presented in the module) in contact with the object within								
2^{nd}-3^{rd} min (61-180 s)	87.5	89.6	90.4	90.6	90.6	86.2	0.80	0.52
4^{th}-5^{th} min (181-300 s)	94.8	92.7	94.7	93.8	93.8	94.7	0.79	0.95
Contacts rabbit-object (n/rabbit)								
1^{st} min (1-60 s)	1.59	1.39	1.36	1.42	1.58	1.34	0.55	0.39
2^{nd}-3^{rd} min (61-180 s)	2.75	2.53	2.49	2.61	2.96	2.19	0.56	0.02
4^{th}-5^{th} min (181-300 s)	3.30	3.11	3.03	2.90	3.60	2.95	0.68	0.02
Total	7.68	7.04	6.91	6.97	8.15	6.51	0.31	0.01

Table 4.9

Corticosterone levels (ng/g) in hard faeces and hair at 65 d of age: effect of floor combination, gender composition and their interaction

	Floor type (F)			Gender composition (G)			P-value			RSD
	Steel/Wire	Steel/Plastic	Wire/Plastic	Females	Males	Mixed	F	G	FxG	
Rabbits (n)	32	32	32	30	30	36				
Corticosterone (ng/g)										
Faeces	46.7	44.9	45.7	47.3	45.0	44.9	0.86	0.71	0.81	13.0
Hair	6.40	5.33	6.02	5.23	5.87	6.65	0.11	0.02	0.62	2.02

In the study of Trocino et al. (2004), rabbits reared on steel slat and wire-net had similar growth performance. Also other authors noticed no substantial difference in growth performance when plastic or rubber floor was compared to wire-net (Abdelfattah et al., 2013; Princz et al., 2009).

The preference of younger animals for the plastic floor rather than for the steel slat or the wire-net could be explained by the higher thermal comfort and by the adaptability of the plastic floor for resting or moving. Steel slat could be cool or more slippery for the animals, especially in the early post-weaning period. Additionally, the distance among steel slats seemed not to promote animals displacements, even if it was not so wide (15 mm). On the other hand, wire-net could be less suitable for young rabbits, compared to the more compact structure that plastic floor could provide. Concerning the interaction between rabbits age and floor type preference, worth mentioning that with the increase of animals age (and size) more and more homogeneous distribution on the modules was noticed, as available space per animal was reduced rendering difficult any intention to express preference for one of the two modules. Similar studies have been also conducted by other researchers to find out rabbits preference for different floor types. In the study of Gerencsѐr et al. (2012) pens containing three floor materials (1/3 plastic-mesh, 1/3 deep litter and 1/3 wire-mesh) were used and rabbits could, freely, pass from one floor part to the other. In accordance with the present thesis, it was noticed that rabbits at the beginning of the trial preferred staying on the plastic floor, but reaching the end of the study this preference attenuated (rabbits on plastic floor: from 70% the 5th week of trial to 52% the 11th) and the use of the other two floors was augmented (wire-mesh: from 23 to 33%; deep litter: from 8 to 14%) until the end of the trial. Similarly, Princz et al. (2008b) evaluated rabbits preference for plastic or wire-net floor when reared at two different stocking densities (12 and 16 animals/m^2). It was observed that rabbits preferred plastic than wire-net, but this was more mild as rabbits were getting older (until 10.5 weeks of age), and with the increase of stocking density more rabbits chose the wire-net. Plastic mesh was, also, preferred by rabbits when they could choose among planked, plastic-mesh, plastic-slat or wire-mesh floor by passing from one cage-block to the other through swing doors (Matics et al., 2003).

Concerning the effect of floor type on behavioural budget time, few differences were noticed. At the first ethogram, rabbits from the steel/wire group spent more time self-grooming which could mean that this floor combination could trigger their attention towards themselves (self-grooming) than their co-specifics (allo-grooming). Probably, this could have been resulted due to a less friendly and comfortable environment; but, in that case, a higher time devoted in total resting would have been expected. Actually, rabbits reared on the steel/wire floor adopted for more time the crouched position during resting than the other groups; although, time spent resting totally was not affected by floor type. So, an explanation is difficult to be given. Resting with body crouched is a prevalent way for rabbits to rest. Animal age, floor type, space availability, environmental temperature and other factors may influence time spent in resting with body crouched or stretched. At the second ethogram, time spent displacing and exploring was higher for the rabbits reared on the steel/wire combination; whereas at the last ethogram, only the time dedicating allo-grooming was higher in the steel/plastic group. Other studies conducted to compare rabbits behaviour reared on different floor types gave inconsistent results. Dal Bosco et al. (2002) noticed that rabbits reared in wire-net pens spent more time resting and less self-grooming and in locomotory behaviours compared to those reared in straw bedded pens, which could have happened because litter was getting dirty more easily than the wire-net, so animals had to devote more time in clearing their fur. Jekkel and Milisits (2009) saw differences in behaviour when they compared rabbits reared on wire-net with those on wire-net combined with straw litter. Rabbits on the combined floor ate, rested, performed comfort and stereotypic behaviours for less time and social behaviours for more time than those on the wire-net floor did. In that case, the lower locomotion of the rabbits reared on the combined floor was attributed in the less comfort of that floor. On the other hand, Trocino et al. (2004) did not observe any difference among rabbits reared on wire-net or steel slat; whereas, in the studies of Princz et al. (2008a) and Abdelfattah et al. (2013) only differences in agonistic behaviours appeared when different flooring was examined (wire mesh vs. plastic-net and wire mesh vs. plastic mat vs. rubber mat, respectively).

Regarding the influence of the floor combinations on rabbits reactivity during the tonic immobility test, little was noticed. Interesting, though, was that all of the animals tested (100%) turned into immobility within three attempts. In the study of Trocino et al. (2013), when rabbits undergone the same test at 42 and 70 d of age, the percentage was lower (92 and 65%, respectively). In the present trial, no difference on the number of attempts made to induce immobility or the duration of the immobility was noticed. Contrary, Trocino et al. (2008) demonstrated that the presence of straw litter, apparently, created a sense of discomfort or insecurity to animals, as during the test the rabbits reared on this floor turned immobile quicker (less attempts) than those reared on wire-net, plastic or steel slat floor. Indeed, the number of attempts needed to immobilize the animal is believed to be positively correlated with fear of man and an underlying stress under rearing conditions (Forkman et al., 2007; Verga, 2000). Of course, it should be taken under consideration the fact that in the present trial the animals were given the opportunity to choose between two floor types, where one of them seemed to give a sense of comfort; at this point, a direct comparison with experiments that studied animals reared on fixed floors (without the opportunity of choosing) should be done with caution.

Contrary, animals reactivity towards a new environmental situation was influenced. The animals reared in the wire/plastic pens had a more positive attitude during the open-field test, as they entered more spontaneously into the arena and performed more active behaviours (moving, running, hopping etc.) compared to the other groups. In the study of Trocino et al. (2008), rabbits reared on wire-net and plastic slat performed more explorative behaviours and rested immobile within the arena for less time than those reared on steel slat or wire-net covered by litter, which was “translated” as a positive reaction to the test. Also, Trocino et al. (2004) noticed that rabbits reared on wire-net performed more explorative behaviour than those reared on steel slat floor. When rabbits reared in conventional bicellular cages were compared with those reared in groups within pens (20-54 animals/pen) with wooden slat floor, rabbits coming from the pens demonstrated less active behaviours (exploration, moving, running etc.) than those reared in the cages (Trial No. 1, present thesis). These behaviours were attributed by the authors to the inappropriate-for-rabbits wooden slat floor, rather than the type of housing *per se*(bicellular cages vs. collective pens).

Effect of gender composition of group: females vs. males vs. mixed group

The increase of aggressiveness, which is usually observed close to the age of sexual maturity, may influence stress level of animals and their productivity. In the present trial, growth performance was not affected by the sexual composition of the groups. Nevertheless, other studies have reported differences in productivity between genders, even if the results do not always match. In details, Di Meo et al. (2003) noticed higher daily weight gain and lower mortality from 49 days to 70 days of age in females group than males and mixed-gender groups, whereas the mixed group resulted having the lowest final weight at 90 days of age. Therefore, rearing in mixed groups was not recommended. Also, Szendrő (2012c) did not suggest single gender-group rearing as it resulted in a higher injuries percentage compared to what it was observed in the mixed-gender groups.

According to Vervaecke et al. (2010), irrespective of gender, hierarchies within small groups (8 animals) start setting up from the tenth week of rearing. In captivity, hierarchies are formed regardless of gender; whereas in nature they are created, separately, among groups of males and females. In the second experiment of the present thesis, differences in lesions score among males and females were noticed, with males having higher damage. In the present trial, differences in aggressions were not noticed, even if the different-among-groups distribution of some behaviours (social and explorative) might indicate that already at the age of 8 weeks (second/third ethogram), the expression of certain behaviours are linked to establish hierarchies within group. Even territoriality may influence aggressiveness among animals. Indeed, interaction among adults becomes even more difficult, mainly when they are reproductively active. Rearing of does in groups resulted in higher injuries rank because of does higher aggressiveness (increased stress) (Szendrő et al., 2012b).

Animals reactivity during the tonic immobility and human-contact tests did not change according to the gender composition. Concerning rabbits response to the open-field test, differences were, mostly, focused among the mixed groups and the single-gender groups, with the former being less active (less locomotory behaviours) and maybe more afraid (stood still for more time). Also reactivity towards the novel object test gave similar results, again with the rabbits reared in the mixed groups being more prudent (less contacts with the object). Probably, mixed rearing provoked higher stress to the animals reducing their confidence to handle with a new situation (novel space/object).

The above mentioned assumption was reinforced by the hair corticosterone levels, which were higher in the mixed groups than the single-gender ones and the male than female groups. Probably, males in the presence of females in the mixed-gender pens became more stressed. Onbaşilar and Onbaşilar (2007) also noticed that gender may influence plasma corticosterone level: males had higher concentration than females when the animals were housed in groups of one, three and five rabbits per cage.

Conclusions

The results obtained showed a clear preference of the rabbits for the plastic slat floor rather than the wire-net and even less for the steel slat. Preference differences tended to fade with the increase of age, probably due to the increase of space requirements: rabbits, in order to satisfy their need for minimum space available, chose to stay on a less preferred floor. Floor combinations did not affect to a great extent rabbits fear level. Indeed, animals reactivity towards man was not influenced, whereas the reactivity toward a new environment it was: animals reared on the most acceptable floor combination (wire-net/plastic) were more keen on exploring the new environment. Rabbits budget time was not influenced by the floor combinations; only few behaviours occupying minimum time (<2% of the time observed) were, somehow, affected.

On the other hand, rearing in mixed groups resulted in more timid reply to the reactivity tests (reactivity towards a new environmental stimulus) and in higher hair corticosterone level. The latter could have been correlated with differences in aggressiveness among animals or body injuries, but this was not observed during the present trial. Lastly, growth performance was not influenced by any of the experimental factors. To conclude, further investigation should be done to assess the possibility for rearing of growing rabbits in single-gender groups.

Conclusions

Rabbits are reared intensively for meat production in wire-net cages in pairs (Italy, Hungary) or in small groups of four to eight animals per cage (other European countries). This housing system has been criticized in view of animal welfare since it is believed to diminish rabbits opportunities for expressing the species-specific behavioural repertoire and to provoke stress and fear to the animals, which may negatively affects their health status and productive performance. Basing on the raising public demand for “welfare-friendly” production techiniques, a strong pressure to producers is going to be exerted to change the methods they currently use. In fact, at the present moment, Northern European Countries are banning the traditional cage systems, whereas the major European producers (i.e. Italy, France, Spain and Hungary) are not yet convinced about the opportunity of changing their system towards pen systems with large groups of rabbits.

Despite the pressure towards a change of the current systems, scientific-based information on the effects of pen housing on rabbits welfare, in view of behaviour, reactivity and stress of the animals, is less frequent; in fact, farmers are hesitant as they believe that such a shift will probably impair growth performance and meat quality traits.

In view of the ongoing effort to pass from the cage to the pen rearing, the present Thesis aimed at evaluating the welfare conditions of rabbits reared collectively in pens under different systems and conditions and with special emphasis on behaviour, reactivity and stress indicators. Productive performance was also controlled to sustain farmers with valuable information on the effects and eventual negative consequences, of the pen housing.

On the base of our results, pen housing may be a succesfull way for growing rabbits giving productive results comparable with those obtained in the current cage systems, but onlyif housing conditions are suitable for the animals.

Surely, rabbits reared in pens and in groups demonstrated a complete, typical-for-the-species behavioural repertoire. The conditions of pen housing tested in the present Thesis, i.e. individual and functional space availability, floor type, gender composition of groups or the presence of enrichment, did not modify the ethogram with regards to the rate and prevalence of the main behaviours when rabbits were in a comfortable condition. Only when some factors, i.e. the wooden slatted floor, were not suitable for rabbits safe displacement, some major effects were noticed in terms of an increase of the resting time at the expense of other important activities (i.e. feeding).

The same result was observed for reactivity of rabbits towards man or new environmental conditions: i.e. the experimental factors tested within pen housing systems modified rabbits reactivity to a weak extent. Once again, when housing conditions were not suitable (i.e. wooden slatted floor) the rabbits reactivity was negatively affected and rabbits were more afraid to face a new situation.

From the methodological point of view and regarding the welfare indicators used, ethogram was always useful to evaluate the general condition of the animals; among reactivity tests, the tonic immobility test and the contact test were less useful to differentiate the experimental groups, whereas the reaction of the animals during the open field test was more evident and consistent with other welfare indicators. Glucocorticoids measurements had a great potentiality even if the results obtained on the different matrices need to be evaluated in a different way taking into account what kind of stress is investigated (acute or crhonic stress).

From the technical point of view, considering the different factors tested in the trials conducted for the present Thesis, we could state that:

- when rabbits do not feel safe while moving,because of an unsuitable pen floor, negative effects are detected on welfare in terms of behaviour, reactivity and stress indicators;
- rabbits show a clear preference for a plastic floor, even if the use of an alternative but suitable floor (like wire net or steel slat) has scarce effects on behaviour, reactivity or stress;
- the reduction of pen stocking density from 16 to 12 animals/m^2 (i.e. from the standard value used in cages to a lower one) does not affect animal welfare, and thus a stocking density of 16 animals/m^2 and a live weight at slaughter within 40-42 kg/m^2 are compatible with both productivity and welfare threshold also in pens. In fact, within pens, the reduction of theindividual available space has a limited effect because of the larger functional space in comparison with cage systems;
- the use of a platform as an enrichment element to stimulate behavioural repertoire of rabbits is successful, also, in view of increasing the functional space without negative consequences on productivity and welfare;

- the use of a tube as an enrichment element needs to be further evaluated because it impairs productivity and, especially, rabbit reactivity and stress level; a higher competition to occupy the tube could be hypothesised as a possible reason;
- all factors which could become limiting at later stages of growth (i.e. available individual and functional surface, gender composition) must be carefully taken into account when establishing the optimum slaughter age for the pen system used, because rabbits stress levels and occurrence of aggressions may quickly increase with age.

In order to encourage producers to abandon the old practices and adopt alternative housing systems, security for their profit must be provided to them. This can be achieved only by associating productivity with welfare indicators during the evaluation of the new systemsand by verifying that both are respected.

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