World Rabbit Sci. 2018, 26: 135-147
doi:10.4995/wrs.2018.7747

BEHAVIOUR AND REACTIVITY OF FEMALE AND MALE RABBITS HOUSED IN COLLECTIVE PENS: EFFECTS OF FLOOR TYPE AND STOCKING DENSITY AT DIFFERENT AGES

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Abstract: At 33 d of age, 376 crossbred rabbits of both genders were housed in sex-mixed groups in 16 open-top collective pens (1.68 m^2) in a factorial arrangement, with 2 types of flooring (wooden slats *vs.* plastic grid, W *vs.* P) and 2 stocking densities (12 *vs.* 16 animals/m^2). Behavioural recordings (time budget), reactivity towards unknown humans (tonic immobility and human approach tests) and environment (open-field and novel object tests) and hair and faeces sampling for corticosterone analysis were performed during the 8th wk of age and at a pre-slaughter age (during the 11-12th wk of age). The effects of age and gender were also taken into account. Rabbits housed in W pens rested more in the crouched position (41.4 *vs.* 35.5% of the observed time) (P<0.001) and showed lower allogrooming (P=0.05), running (P<0.01) and biting pen elements (P=0.01) than those housed in P pens. The percentage of rabbits that interacted with humans during the human approach test (24.0 *vs.* 48.1% of rabbits in the pen; P<0.01) and number of rabbit-object contacts during the novel object test (on av. 50.4 *vs.* 87.2; P<0.001) were lower for rabbits in W pens compared to those in P pens. During the open-field test, the percentage of rabbits that spontaneously entered the arena was lower for rabbits from W pens than for those from P pens (60.0 *vs.* 72.5%; P<0.05). Finally, the hair corticosterone level was higher in the former than in the latter rabbits (on av. 14.0 *vs.* 12.5 ng/g; P<0.05). As the stocking density increased, only the time spent resting increased (66.7 to 69.1% observed time; P<0.01), and the percentage of rabbits that spontaneously entered the arena during the open-field test decreased (73.8 to 58.8%; P<0.01). When age increased, the rabbits were less active at the reactivity tests and interacted less with an unknown object or person. Differences according to gender were weak. In conclusion, the wooden slatted floor challenged the welfare of growing rabbits as it constrained their movement, conditioned their reactivity towards a new environment, and increased their stress level, whereas the increase in stocking density did not impair rabbit welfare.

Key Words: collective housing, time budget, fear, stress, gender, age.

INTRODUCTION

Public opinion is demanding animal-friendly rearing systems for all species kept for farming purposes and, in particular, the European Parliament is pushing to ban cages for growing rabbits (European Parliament, 2017). Collective pen housing represents the most feasible alternative to current systems, as it satisfies the social nature of rabbits and offers space for movement and different activities (Trocino and Xiccato, 2006). In fact, in recent years Northern European Countries have been promoting this change of housing systems, whereas the Mediterranean Countries (i.e., France, Italy, and Spain) are late starters.

Initially, collective pens with large group sizes (>10 rabbits/pen) have raised several concerns because they can impair performance and meat quality compared with conventional small group systems (Maertens and Van Oeckel, 2001; Princz *et al.,* 2009; Xiccato *et al.,* 2013). At present, more information is available on the technical standards

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https://doi.org/10.4995/wrs.2018.7747

for housing and management of rabbits in collective systems, which permits optimal animal performance under commercial conditions. Aggression among animals increases under collective systems (Lambertini *et al.,* 2005; Szendrő *et al.,* 2009), and this could affect animal welfare. Moreover, several factors (i.e., pen floor type, enrichment, stocking density, group size, sex-composition of groups, or slaughter age) may affect the welfare of rabbits reared collectively, but their role has not yet been fully elucidated (Szendrő and Dalle Zotte, 2011).

Several indicators are required to evaluate animal welfare, other than productive indicators. A primary indicator to assess changes in behavioural patterns is the behavioural time budget (Morisse *et al.,* 1999; Keeling and Jensen, 2009). Other indicators are reactivity tests to evaluate the emotional state of animals and their adaptation to the environment they live in (Forkman *et al.,* 2007; Buijs and Tuyttens, 2015). In fact, because of environment and management, farm animals may experience fear and anxiety, negative emotions usually included in the assessment of animal welfare, which could be assessed by reactivity tests (Forkman *et al.,* 2007; Waiblinger, 2009). Finally, glucocorticoid concentrations are used as physiological indicators of welfare to evaluate both acute and chronic stress (Keeling and Jensen, 2009; Buijs *et al.,* 2011a; Prola *et al.,* 2013).

The goal of the present study was to evaluate whether and how housing conditions, namely the floor type (wooden slats *vs.* plastic grid) and the stocking density (12 *vs.* 16 animals/m^2), might affect the behaviour, reactivity, and stress level of rabbits housed in collective pens in large groups (20-27 rabbits). The effects of age (8 wk *vs.* pre-slaughter age, 11-12 wk) and gender were also evaluated.

MATERIAL AND METHODS

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

Animals and housing

At weaning (33 d of age), a total of 376 Hyplus rabbits (Hypharm, Groupe Grimaud, Roussay, France) of both genders were selected from healthy litters and moved to the experimental stable of the University of Padova. Temperatures varied between 18 and 25°C and a natural photoperiod (11-13 h daylight) was used. On arrival, rabbits were individually identified by ear mark. Then, the animals were housed in 16 open-top pens (1.20×1.40 m, i.e., 1.68 m^2) at 2 stocking densities (12 and 16 animals/m^2). Half of the pens were equipped with a wooden slatted floor (untreated fir; width of the slat: 8 cm; distance between the slats: 3 cm) originally present in the same pens; the other half were equipped with a plastic grid (rectangular holes: 1.0×7.0 cm; distance between the holes: 0.7 cm) commercially available (Meneghin s.r.l., Povegliano, Italy) (Figure 1). 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 4 experimental groups: W12 (wooden slatted floor, 12 animals/m^2): 80 rabbits in 4 pens with 20 animals/pen; W16 (wooden slatted floor, 16 animals/m^2): 108 rabbits in 4 pens with 27 animals/pen; P12 (plastic grid floor,

Figure 1: Collective pens with plastic grid (left) and wooden slatted floor (right).

12 animals/m^2): 80 rabbits in 4 pens with 20 animals/pen; P16 (plastic grid floor, 16 animals/m^2): 108 rabbits in 4 pens with 27 animals/pen.

Within each experimental group, half of the animals (2 pens) were slaughtered at 76 d of age, and the remaining animals (2 pens) were slaughtered at 83 d of age to evaluate if the increase of age could modify the behaviour, growth performance and slaughter results of rabbits (Trocino *et al.,* 2015).

The animals had *ad libitum* access to fresh water through nipple drinkers and to a pelleted diet through feeders for manual distribution. Further details of animal management and results of growth performance, sanitary status, commercial slaughter, and carcass and meat quality recordings are available in Trocino *et al.* (2015).

Behavioural evaluation

The behaviour of the rabbits was video-recorded for 24 h during the 8th wk of age (at 50 d of age, all pens) and at pre-slaughter age, i.e., 11-12 wk of age (at 71 d, 8 pens housing rabbits to be slaughtered at 76 d; at 78 d of age, the remaining 8 pens housing rabbits to be slaughtered at 83 d). During the night, a minimal light (approximately 15 lux) was used to avoid disturbing the rabbit activities. Two minutes were analysed for each hour of observation from each pen. The following behaviours were analysed and expressed as a percentage of the observed time: resting (crouched body, with the 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, allogrooming, feeding, drinking, moving, running, standing still, biting and sniffing (Morisse *et al.,* 1999; Dal Bosco *et al.,* 2002; Trocino *et al.,* 2013). The occurrence (n) of rearing, hops, aggressive interactions and stereotypic behaviours was also recorded.

Tonic immobility and human approach testing

The tonic immobility test and the human approach test were used to measure the animal fear level towards man (Ferrante *et al.,* 1992; Forkman *et al.,* 2007; Verwer *et al.,* 2009). The tonic immobility test was performed on a total of 160 rabbits: 80 rabbits at the 8th wk of age (53 d; 5 animals×16 pens), 80 rabbits at pre-slaughter age, i.e., 11-12 wk of age (40 rabbits at 74 d, 5 animals×8 pens to be slaughtered at 76 d; 40 rabbits at 81 d, 5 animals×8 pens to be slaughtered at 83 d). At each age, the test used different animals. The test was performed in an adjacent room in the same barn where the pens were located. The operator took the rabbit out of the pen and induced 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 3 attempts to induce immobility were used, and rabbits were left in the immobile condition for no more than 180 s. The number of attempts necessary to induce immobility and the total duration of immobility were recorded for each rabbit.

The human approach test was performed during the 8th wk of age (at 49 d of age, all pens) and at pre-slaughter age, i.e., 11-12 wk of age (in eight pens at 70 d and in the remaining eight pens at 77 d). An operator unfamiliar to the animals opened each pen and sat at the entrance of it placing his arm on the centre of the pen a few centimetres above the floor (at the animals' withers height). The animals' reactions were video-recorded for 3 min, and the latency until the first contact with the operator and the number of rabbits that touched or sniffed him were recorded and expressed as a percentage of the rabbits in the pen (Csatádi *et al.,* 2007; Verwer *et al.,* 2009).

Open-field test

The open-field test was used to investigate the reactivity and fear level when the animals faced a new environment, and should mimic the risk of predation for those species that in nature normally hide (Meijesser *et al.,* 1989; Forkman *et al.,* 2007; Buijs and Tuyttens, 2015). The open-field test was performed on a total of 160 rabbits: 80 rabbits at the 8th wk of age (54 d, 5 animals×16 pens) and 80 rabbits at pre-slaughter age, i.e., 11-12 wk of age (40 rabbits at 75 d, 5 animals×8 pens to be slaughtered at 76 d; 40 rabbits at 82 d, 5 animals×8 pens to be slaughtered at 83 d). The rabbits had not been previously submitted either to the tonic immobility testing or the open-field test.

The test was performed simultaneously in 2 arenas (1.5×1.5 m) with 0.80-m-high wooden walls and plastic floors divided into 9 numbered squares. The arenas were located in an adjacent room in the same barn where the animals were kept. The total duration of each test was 10 min per animal. Every rabbit was put in a closed wooden box

Table 1: Behaviours during the open-field test (Meijesser *et al.,* 1989; Ferrante *et al.,* 1992; Trocino *et al.,* 2013).

Definition	Description
Total displacements	Number of squares that the rabbit crossed in the arena
Central displacements	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 the squares
Running	Time spent in running among the squares
Exploration	Time spent moving with forelegs or standing while sniffing and looking around inside the same square
Escape attempts	Number of rapid runs towards the corners of the arena
Hops	Number of times the rabbit completely displaced its body by a hop
Standing still	Time the rabbit spent still with its fore and hind legs unstretched and on the ground
Rearing	Number of times the rabbit reared up on its hind legs
Grooming	Time spent self-grooming
Digging	Time spent digging inside the arena
Biting	Time spent biting elements of the pen
Resting	Time spent inactive with the body touching the floor and fore and/or hind legs stretched on the floor
Defecation	Number of times the rabbit defecated
Urination	Number of times the rabbit urinated

(22×30 cm×30 cm-high) connected to the arena by a sliding door. After 1 min, the sliding door was opened. The number of attempts the rabbit made and the time (latency) taken to enter the arena were recorded for 1 min. 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 8 min. The behaviours that were considered during the test are described in Table 1 (Meijesser *et al.,* 1989; Ferrante *et al.,* 1992; Trocino *et al.,* 2013).

Novel object test

The novel object test was used to measure reactivity towards a new stimulus (Verwer *et al.,* 2009). The test was performed at the 8^{th} wk of age (52 d, all pens) and at pre-slaughter age, i.e., 11-12 wk of age (in 8 pens at 73 d and in the remaining 8 pens at 80 d). To avoid any habituation effect, 2 different objects, unknown to the animals, were used: a half-full bottle of water (1.5 L) anchored by the cap with an iron chain and dropped from the roof in the centre of each pen to a few centimetres above the floor; and a litter nest, made of galvanised iron and placed in the centre of each pen. In each pen, a different object was used in the first and in the second test. The behaviour in each pen was video-recorded for 10 min, and the total number of rabbit-object contacts was measured at minute 1, 3, 7, and 10 regardless of the single rabbit touching the object (Verwer *et al.,* 2009).

Corticosterone determination in hard faeces and hair

Individual sampling of hard faeces and hair on the animals submitted to the tonic immobility test took place immediately after the test. Accordingly, a total of 160 rabbits were used: 80 rabbits at the 8^{th} wk of age (53 d, 5 animals×16 pens) and 80 rabbits at pre-slaughter age, i.e., 11-12 wk of age (40 rabbits at 74 d, 5 animals×8 pens to be slaughtered at 76 d; 40 rabbits at 81 d, 5 animals×8 pens to be slaughtered at 83 d).

Some hard faeces pellets were collected between 16.00 and 18.00 h directly from each animal by applying gentle pressure to the perianal area. Hair samples were collected by gently pulling hair from the back and hind legs. The corticosterone levels were measured by microtitre radioimmunoassay using species-specific antibodies (Biogenesis, Poole, England, UK), as detailed by Simontacchi *et al.* (2009) and after steroid extraction (Trocino *et al.,* 2014).

Statistical analysis

The behavioural data were analysed with a mixed model that used pen floor (wooden slats *vs.* plastic grid), stocking density (12 *vs.* 16 rabbits/m^2), age (8 wk *vs.* pre-slaughter age), and their interactions as fixed effects and observation hour as a random effect. The data for the 2 pre-slaughter ages (11 and 12 wk of age) were merged after testing showed they were not different. The PROC GLIMMIX of SAS software (2013) was used. Data from the same pens were treated as repeated measures. An underlying Poisson distribution was assumed for data expressed as a percentage of observed time.

In the case of the tonic immobility and open-field tests, reactivity data were analysed by PROC GLIMMIX with pen floor, stocking density, age, gender and their interactions as fixed effects and pen as a random effect. In the case of the human approach and the novel object tests, data were analysed with the same model without the effects of gender and pen. In the case of the novel object tests, the model also included the effect of the object. The data on the percentage of sensitive animals for the tonic immobility test and animals entered in the open-field test were analysed by PROC CATMOD with pen floor, stocking density, age, gender and their interactions as fixed effects.

The data on corticosterone levels in faeces and hair were analysed using PROC MIXED with pen floor, stocking density, age, gender and their interactions as fixed effects and pen as a random effect.

Differences among means with P<0.05 were accepted as representing statistically significant differences. Only significant interactions were included in tables and presented in results and discussion.

RESULTS

Effect of floor type: wooden slatted floor vs. plastic grid

Rabbits reared on the wooden floor spent more time resting in the crouched position (41.5 *vs.* 35.3% of observed time; in average of the 2 ages) and less time in the stretched position (27.2 *vs.* 31.9%) (P<0.001) compared to those reared on the plastic floor (Table 2). Concerning less frequently observed behaviours, the rabbits on the wooden floor spent less time on allogrooming (P=0.05), running (P<0.01) and biting pen elements (P=0.01) compared to rabbits kept on the plastic floor.

The behaviour at the tonic immobility test was not affected by the floor type (Table 3), whereas during the human approach test, the percentage of animals that touched the operator was lower in rabbits housed in pens with a wooden floor than in those in pens with a plastic floor (P<0.01) (Figure 2).

During the open-field test, the percentage of rabbits that spontaneously entered the arena was lower for rabbits kept on the wooden floor than for those kept on the plastic floor (60.0 *vs.* 72.5%, in average of the 2 ages; P<0.05) (Table 4b). The former rabbits also spent more time in cautious exploration (P<0.01) and grooming (P<0.01), and they crossed the arena centre fewer times (P<0.001) than the rabbits housed on the plastic floor. Nevertheless, the behaviour of rabbits from pens with different floors changed depending on the animal age: the rabbits housed in pens with plastic floor at the first open-field test displayed the highest values for active behaviours, i.e., number of total and central displacements, time spent on running and grooming (Table 4b). Moreover, the rabbits housed in the pens with plastic floor at pre-slaughter age displayed the highest values for standing still (Table 4b).

During the novel object test, the number of rabbits-objects contacts was always lower in pens with a wooden floor than in those with a plastic floor (P<0.001) (Table 5).

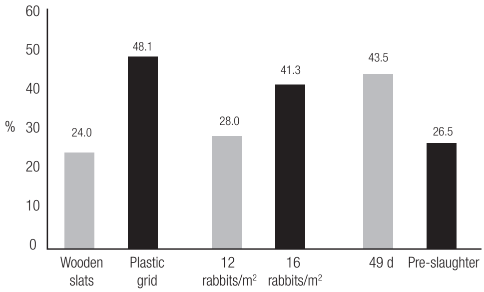

Figure 2: Effect of floor type, stocking density and age on the percentage of rabbits (% of rabbits in the pen) that touched the operator during the human approach test.

Table 2: Behaviours (% of observed time) of growing rabbits housed in collective pens: effect of floor type, stocking density, and age.

	Floor (F)				Stocking density (rabbits/m²) (D)				P-value				
	Plastic grid	Wooden slats	Plastic grid	Wooden slats	12	16	12	16					
Age (A)	8 wk	8 wk	Pre-slaughter	Pre-slaughter	8 wk	8 wk	Pre-slaughter	Pre-slaughter	F	D	A	A×F	A×D
Observations (n)	192	192	192	192	192	192	192	192					
Feeding (%)	11.4	10.7	7.00	6.89	10.9[c]	11.2[c]	7.44[b]	6.48[a]	0.23	0.09	<0.001	0.32	0.001
Drinking (%)	1.74	1.88	1.47	1.44	1.89	1.74	1.47	1.45	0.69	0.50	<0.001	0.40	0.52
Total resting (%)	62.5	63.5	71.7	73.9	62.2	63.8	71.2	74.4	0.05	<0.01	<0.001	0.38	0.31
Body stretched (%)	30.8	25.6	32.9	28.7	29.4	26.9	32.9	28.7	<0.001	<0.001	<0.001	0.08	0.09
Body crouched (%)	31.8	37.8	38.8	45.1	32.8[a]	36.6[b]	38.5[c]	45.5[d]	<0.001	<0.001	<0.001	0.34	0.01
Self-grooming (%)	16.7	16.7	14.4	14.4	17.1	16.3	15.1	13.5	0.67	0.001	<0.001	0.69	0.09
Allogrooming (%)	0.37	0.20	0.17	0.16	0.29	0.25	0.18	0.15	0.05	0.29	<0.01	0.10	0.84
Moving (%)	1.55[bc]	1.67[c]	1.34[b]	1.07[a]	1.58	1.64	1.29	1.11	0.31	0.44	<0.001	0.01	0.14
Running (%)	0.13	0.03	0.04	0.004	0.007	0.05	0.02	0.01	<0.01	0.36	0.02	0.61	0.69
Biting (%)	0.14	0.05	0.13	0.08	0.08	0.07	0.13	0.09	0.01	0.48	0.22	0.17	0.56
Sniffing (%)	5.29	5.71	3.42	3.30	5.84	5.17	3.44	3.28	0.64	0.06	<0.001	0.12	0.30

[a,b,c]Means in a row within main effect (floor type or stocking density) with different superscript letters are statistically different ($P<0.05$).

Table 3: Response to the tonic immobility test of growing rabbits housed in collective pens: effect of floor type, stocking density, age and gender.

	Floor (F)		Stocking density (rabbits/m²) (D)		Age (A)		Gender (G)		*P*-value				
	Plastic grid	Wooden slats	12	16	8 wk	Pre-slaughter	Female	Male	F	D	A	G	A×G
Rabbits (n)	80	80	80	80	80	80	87	73					
Sensitive rabbits[1] (%)	93.8	90.0	92.5	91.3	92.5	91.3	89.7	94.5	0.38	0.73	0.92	0.23	0.74
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
Immobility[2] (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

[1]Percentage of rabbits turned immobile within 3 attempts.

[2]Probability of the interaction age×gender, $P<0.001$: 53.9 s and 75.3 s in females and males at 53 d, and 67.8 s and 40.5 s in females and males before slaughter, respectively.

The type of object influenced rabbit reaction: the nest was largely preferred to the bottle (+85% rabbits-objects contact) (P<0.001) (Table 5).

The hair corticosterone level was higher in the rabbits reared on the wooden floor compared to those reared on the plastic one (P<0.05), whereas the faeces corticosterone level did not change (Table 6).

Effect of age

As rabbits became older, the time spent feeding (11.1 to 6.95% on average for the pens with different floors and stocking densities; P<0.001), drinking (1.81 to 1.46%; P<0.001), self-grooming (16.7 to 14.4%; P<0.001) and sniffing (5.50 to 3.36%; P<0.001) decreased. Among the less observed behaviours, the time spent allogrooming (P<0.01), running (P<0.001) and moving (P<0.05) were similarly reduced (Table 2). Conversely, the time spent resting increased (63.0 to 72.8%; P<0.001), especially with a crouched body (34.8 to 42.0%; P<0.001).

The duration of immobility decreased with the increase of age (P<0.001) (Table 3). During the human approach test, the percentage of animals that touched the operator was lower at the pre-slaughter age than at the 8th wk of age (P<0.001) (Figure 2).

At the open-field test, the percentage of rabbits entering the arena spontaneously decreased according to age (76.3 to 53.3% from 50 d to pre-slaughter age) (Table 4b). Moreover, as age increased, the number of total displacements and the time spent in cautious exploration, running and grooming decreased (P<0.01) (Table 4b).

During the novel object test, the number of rabbits-objects contacts was lower in older rabbits compared to the younger ones (P<0.001) (Table 5). Age did not affect hair or faeces corticosterone levels (P>0.05) (Table 6).

Effect of gender

The duration of immobility was affected by the gender, as it was lower in females than in males at 8 wk of age (53.9 *vs.* 75.3 s), but higher in females than males at pre-slaughter age (67.8 *vs.* 40.5 s) (probability of the interaction age×gender, P<0.001) (Table 3).

During the open-field test, females exhibited more active behaviours (total and central displacements, running, grooming) compared to males (P<0.001) (Table 4a). Conversely, males spent more time in cautiously exploring their surrounding and standing still compared to females (P<0.001). Females at 8 wk of age were more active than the other groups, displaying the highest values for total displacements, running time and grooming time (significant interaction gender x age; P<0.001) (Table 4a). Males showed the highest standing still time at pre-slaughter age compared to the other groups (significant interaction gender x age; P<0.05).

Gender did not affect hair or faeces corticosterone levels (P>0.05) (Table 6).

Table 4a: Behaviours of growing rabbits housed in collective pens during the open-field test: effect of gender and age.

	Gender (G)				*P*-value	
	Female	Male	Female	Male		
Age (A)	8 wk	8 wk	Pre-slaughter	Pre-slaughter	G	A×G
Rabbits (n)	40	40	40	40		
Entered animals[1] (%)	76.2	76.3	60.5	52.4	0.72	0.88
Latency (s)	30.3^b	24.3^a	26.0^a	30.3^b	0.43	<0.001
Total displacements (n)	47.4^c	30.5^a	37.6^b	30.5^a	<0.001	<0.001
Central displacements (n)	1.68	0.69	1.53	0.68	<0.001	0.78
Exploration (s)	379^b	382^b	355^a	375^b	<0.001	0.01
Movement (s)	30.5	26.5	29.2	24.8	<0.001	0.78
Running (s)	16.6^c	8.73^b	3.15^a	3.39^a	<0.001	<0.001
Stay still (s)	33.8^a	37.0^b	47.1^c	58.1^d	<0.001	0.03
Grooming (s)	6.81^c	2.86^b	1.90^a	1.83^a	<0.001	<0.001
Digging (s)	0.20	0.18	1.28	0.61	0.07	0.13

[1]Percentage of rabbits spontaneously entering the arena. ^{a,b,c}Means in a row within main effect (floor type or stocking density) with different superscript letters are statistically different (P<0.05).

Table 4b: Behaviours of growing rabbits housed in collective pens during the open-field test: effect of floor type, stocking density, and age.

	Floor (F)				Stocking density (rabbits/m²) (D)				*P*-value				
	Plastic grid	Wooden slats	Plastic grid	Wooden slats	12	16	12	16					
Age (A)	8 wk	8 wk	Pre-slaughter	Pre-slaughter	8 wk	8 wk	Pre-slaughter	Pre-slaughter	F	D	A	A×F	A×D
Rabbits (n)	40	40	40	40	40	40	40	40					
Entered animals[1] (%)	92.5	60.0	52.5	60.0	85.0	67.5	62.5	50.0	<0.05	<0.01	<0.01	<0.01	0.38
Latency (s)	27.9	26.4	27.7	28.4	27.6^{ab}	26.6^{a}	25.5^{a}	30.8^{b}	0.77	0.19	0.38	0.34	<0.01
Total displacements (n)	42.8^{b}	33.8^{a}	30.9^{a}	37.2^{b}	41.0^{b}	35.2^{ab}	34.2^{a}	33.6^{ab}	0.83	0.53	<0.001	<0.001	0.01
Central displ. (n)	2.79^{c}	0.41^{a}	1.85^{b}	0.56^{a}	1.60^{b}	0.72^{a}	1.04^{b}	0.99^{ab}	<0.001	0.25	0.73	0.03	<0.01
Exploration (s)	369^{b}	392^{c}	341^{a}	391^{c}	373	388	360	370	<0.01	0.33	<0.001	<0.001	0.54
Movement (s)	26.1	31.0	24.2	29.9	30.8	26.2	29.5	24.5	0.03	0.07	0.60	0.68	0.83
Running (s)	20.5^{d}	7.06^{c}	2.42^{a}	4.41^{b}	16.9^{c}	8.54^{b}	2.67^{a}	4.00^{a}	0.31	0.60	<0.001	<0.001	<0.001
Standing still (s)	39.2^{b}	32.0^{a}	70.9^{c}	38.6^{b}	38.0^{ab}	33.0^{a}	54.9^{c}	49.8^{b}	0.02	0.60	<0.001	<0.001	0.39
Grooming (s)	5.49^{c}	3.55^{b}	3.28^{b}	1.06^{a}	5.86	3.33	2.10	1.66	<0.01	0.13	<0.001	<0.01	0.10
Digging (s)	0.13	0.28	0.47	1.63	0.26^{a}	0.14^{a}	0.79^{b}	0.98^{b}	0.08	0.76	<0.001	0.34	0.05

[1]Percentage of rabbits spontaneously entering the arena.

[a,b,c,d]Means in a row within main effect (floor type or stocking density) with different superscript letters are statistically different ($P<0.05$).

Table 5: Number of rabbit-object contacts during the novel object test in growing rabbits housed in collective pens: effect of floor type, stocking density, age, and object.

	Floor (F)		Stocking density (rabbits/m²) (D)		Age (A)		Object (O)		P-value			
	Plastic grid	Wooden slats	12 rabbits/m²	16 rabbits/m²	8 wk	Pre-slaughter	Bottle	Nest	F	D	A	O
Pens (n)	16	16	16	16	16	16	16	16				
Contacts (n)												
Minute 1	16.1	9.76	13.8	12.0	15.3	10.5	9.11	16.7	<0.001	0.15	<0.01	<0.001
Minute 4	23.0	13.7	19.4	17.3	20.3	16.3	13.9	22.8	<0.001	0.31	<0.01	<0.001
Minute 7	25.7	14.0	21.4	18.3	22.3	17.4	14.8	24.9	<0.001	0.16	0.04	<0.001
Minute 10	22.4	12.9	18.7	16.6	20.1	15.2	10.4	24.9	<0.01	0.42	0.01	<0.001

Table 6: Corticosterone levels (ng/g) in hard faeces and hair of growing rabbits housed in collective pens: effect of floor type, stocking density, age, and gender.

	Floor (F)		Stocking density (rabbits/m²) (D)		Age (A)		Gender (G)		P-value				RMSE
	Plastic grid	Wooden slats	12 rabbits/m²	16 rabbits/m²	8 wk	Pre-slaughter	Females	Males	F	D	A	G	
Rabbits (n)	32	32	32	32	32	32	32	32					
Hair (ng/g)	12.5	14.0	12.8	13.7	13.5	13.0	13.5	13.0	<0.05	0.16	0.52	0.52	2.70
Faeces (ng/g)	40.1	43.9	42.9	41.2	44.4	39.7	41.6	42.4	0.16	0.54	0.09	0.77	10.7

RMSE: Root mean square error.

Effect of stocking density

When the stocking density increased from 12 to 16 animals/m^2, the rabbits reduced the time spent self-grooming (16.1 *vs.* 14.9%; P<0.001); conversely, they increased total resting time (66.7 *vs.* 69.1%; P<0.01) and time spent resting in the crouched position (35.7 *vs.* 41.1%; P<0.001) (Table 2). Significant interactions between age and stocking density were recorded for time spent in feeding (with the lowest value in pens with 16 rabbits/m^2 at pre-slaughter age; P=0.001) and for the time spent resting in the crouched position (with the highest value in pens with 16 rabbits/m^2 at pre-slaughter age; P=0.01) (Table 2).

The duration of immobility decreased with the increase of stocking density (P<0.01) (Table 3), whereas no significant difference was measured at the human approach test (Figure 2).

At the open-field test, the percentage of rabbits spontaneously entering the arena decreased according to stocking density (73.8 to 58.8% from pens at 12 rabbits/m^2 to pens at 16 rabbits/m^2; P<0.01) (Table 4b). The highest value for the latency to enter the open-field arena was recorded in rabbits kept in pens with 16 rabbits/m^2 at pre-slaughter age (probability of the interaction stocking density×age; P<0.01) (Table 4b). In contrast, the highest values for total displacements, central displacements and running time were measured in rabbits kept in pens at 12 rabbits/m^2 at 50 d of age (Table 4b).

Stocking density did not affect the results of the novel object test (Table 5) or the hair or faeces corticosterone levels (P>0.05) (Table 6).

DISCUSSION

Effect of floor type: wooden slatted floor vs. plastic grid

Performance data collected during the trial showed that the wooden slatted floor had a clear negative effect on the production results (growth, feed intake, conversion index and carcass traits) of growing rabbits compared to the plastic grid (Trocino *et al.,* 2015). The data in the present paper confirm that animal welfare was also challenged, based on the measured indicators.

In fact, the rabbits reared on the wooden floor were less active, spent less time running and biting elements of the pen, and rested more in the crouched position than rabbits kept on the plastic grid. In fact, the rabbits moved across and explored the pens less because they slipped into the gaps among the wooden slats: the 3-cm distance between the wooden slats used was too large to safely support rabbit movement, especially in the case of small young animals. This likely accounted for their behaviour during the reactivity tests. The rabbits in pens with a wooden floor showed less interaction with humans or objects during the human approach and the novel object tests, probably because they were less prone to move across the pens. The difference in the rabbits-objects interaction according to the two types of flooring persisted regardless of the type of object used in the novel object test and the greater attraction rabbits had for the nest compared with the bottle. Rabbits reared on the wooden floor also hesitated more to enter the arena during the open-field test and exhibited a less active aptitude (Meijesser *et al.,* 1989; Trocino *et al.,* 2013) than those kept in the pens with the plastic grid, especially when young. The corticosterone levels in the hair confirmed that rabbits reared on the wooden slatted floor had a higher stress level compared to those reared on the plastic grid. In fact, based on its accumulation pathways, corticosterone in hair may measure chronic stress in rabbits, as occurs in other species (Cook, 2012). Nevertheless, this did not affect reaction towards man during the tonic immobility test. Indeed, the duration of immobility is believed to be positively correlated with fear level and negatively correlated with the number of attempts required to achieve immobility (Ferrante *et al.,* 1992; Forkman *et al.,* 2007).

Other studies did not find relevant effects of the floor type on rabbit time budget or behaviour in reactivity tests when no objective constraint to movement was evident (Trocino *et al.,* 2004; Princz *et al.,* 2008) or when 2 types of floor (wire net or wire net floor covered with straw) were available in the same cage and the animals had the option of choosing the place to rest (Morisse *et al.,* 1999). In contrast, some studies reported differences in the time spent in comfort behaviours and locomotor activity when the floor was completely covered with straw (Dal Bosco *et al.,* 2002), which has been proven undesirable to rabbits (Morisse *et al.,* 1999; Orova *et al.,* 2004). Under similar uncomfortable conditions (straw-bedded wire floor), Trocino *et al.* (2008) found that rabbits exhibited more fearful behaviours (i.e., standing still) in the open-field test and were more fearful towards man. The rabbits required a lower number of attempts to become immobile in the tonic immobility test compared to rabbits kept on other floors (plastic slat and wire net).

Effect of stocking density

Under intensive systems, stocking density is usually around 16 rabbits/m^2. Indeed, the threshold currently recommended by EFSA (2005) is 40 kg live weight/m^2 at slaughter to preserve growth performance and animal welfare when rabbits are kept in small groups. In the present trial, the stocking density ranged from 32.7 to 42.2 kg live weight at slaughter/m^2 in the pens with 12 and 16 rabbits/m^2, respectively, thus overreaching the recommendations by approximately 5% in the latter case. On the basis of tested indicators and under our conditions, such an increase did not affect the welfare of rabbits housed in collective pens in large groups.

When Buijs *et al.* (2011b) increased stocking density by decreasing pen size while using a stable group size, the reduction in space increased sternal lying (i.e., abdomen in contact with the floor), which was considered a filling behaviour and not a comfort behaviour. Nevertheless, we found that the time spent in the stretched position decreased (and that in the crouched position increased) during resting in cases of reduced space availability (due to increased stocking density or increased age/size of animals) and under uncomfortable conditions (wooden slatted floor) (present trial; Trocino *et al.,* 2014). Under our conditions, this difference in resting behaviour was not likely to affect the general welfare status of the animals, as the rabbit reactivity was scarcely (open-field test) or not affected (human approach and novel object tests). Moreover, the increase in stocking density did not affect the corticosterone levels in the hair or faeces of growing rabbits, whereas their reactivity towards man was improved (reduced immobility duration), as already observed under similar housing conditions (Trocino *et al.,* 2014). The highest latency time to enter the arena of the open-field test measured at the pre-slaughter age in rabbits kept in pens with 16 rabbits/m^2 might be explained by the low motivation the rabbits had to reinstate contact with conspecifics (Buijs and Tuyttens, 2015), rather than fear towards a new environmental situation. In fact, at the end of the trial, aggression among animals was more pronounced in the pens with 16 animals/m^2 than in those with 12 rabbits/m^2, as proven by differences in the rate of injured rabbits (26.2 *vs.* 8.2%, respectively; P<0.001) (Trocino *et al.,* 2015). According to Szendrő *et al.* (2009), behaviours associated with establishing social hierarchy or oncoming sexual maturation likely explain aggression among animals.

In the case of rabbits kept in groups in free range on the ground, the reduction in stocking density from 16 to 12 rabbits/m^2 improved the reactivity of the rabbits, which were more active during the open-field test (Verga *et al.,* 1994).

Effect of age

In accordance with other studies (Morisse and Maurice, 1997; Dal Bosco *et al.,* 2002; Buijs *et al.,* 2011b), our rabbits spent most of their time resting, self-grooming, feeding and drinking. As age increased, the rabbits decreased their activity by reducing time spent on feeding, comfort behaviours (self and allogrooming) and exploration (sniffing, moving, running), whereas they increased resting time, especially with a crouched body. Under different housing conditions (small group size), other authors found decreased feeding time when age increased (Morisse *et al.,* 1999; Martrenchar *et al.,* 2001; Trocino *et al.,* 2013).

When age increased, the rabbits were also less active in the reactivity tests, showing less interest towards an unknown object or person in the pen as well as less sensitivity to the tonic immobility test (lower duration of immobility). In the present trial, at the 2 ages, objects and persons were different for the same pen in the novel object test and different animals were submitted to the immobility and the open-field tests. Thus, the behaviour of rabbits did not depend on any habituation to the tests, which would have decreased the fear level (Forkman *et al.,* 2007), as was hypothesised when the same rabbits were tested at 2 different ages (Trocino *et al.,* 2013). According to Buijs and Tuyttens (2015), motivations other than fear (exploratory and social ones) accounted for the reduction in locomotor activity during the open-field test that they observed over consecutive tests. We hypothesised that the reduced activity (time budget and reactivity tests) depended on a reduction in the motivation for exploration as age increased. Moreover, under our conditions, frequent and careful manipulation of the animals during the trial might have reduced fear level during the tonic immobility or the human approach tests as age increased (Csatádi *et al.,* 2005; Verwer *et al.,* 2009). Finally, the stress level did not change with age, as measured by corticosterone in the hair and faeces. In contrast, Trocino *et al.* (2014) found that hair corticosterone levels increased from 63 to 70 d of age in group-housed rabbits.

Effect of gender

In commercial farms, growing rabbits are housed in mixed groups (males and females) due to little sexual dimorphism and an early slaughter age (Hernández and Dalle Zotte, 2010). The small and non-relevant differences in growth performance and slaughter results do not justify the separation of rabbits by gender when they are maintained under standard housing conditions (i.e., bicellular cages or small cages with 4-6 rabbits) or pen housing systems (Trocino *et al.,* 2015). On the other hand, aggression has been found to be more severe among males than females, especially when sexual maturity approaches and rabbits are reared in large groups (Bigler and Oester; 1996; Verga *et al.,* 2007). Di Meo *et al.* (2003) observed a higher occurrence of injuries in rabbits kept in mixed-sex pens (30 rabbits per pen) compared with rabbits in single-sex pens. In the conditions of the present trial, with mixed pens, the rate of injured rabbits was noticeably higher among males than females (25.8 *vs.* 11.3%; $P \leq 0.001$) at the end of the trial (Trocino *et al.,* 2015).

It is not clear if this result depended on the greater aggressiveness of the females towards the males or the higher aggressiveness among the males approaching sexual maturity. How this affected animal welfare is not clear on the basis of the results of the reactivity tests. In fact, the males at pre-slaughter age showed more fearful behaviour (the highest values of standing still) towards a new environment compared to young males and females at both ages. However, the same animals were less fearful towards humans (lowest immobility duration) in the tonic immobility test. Once again, motivations other than fear (Buijs and Tuyttens, 2015) and, specifically, low motivation for social reinstatement, may explain the behaviour of older males during the open-field test.

CONCLUSIONS

In comparison with a plastic grid, the wooden slatted floor used in the present trial challenged the welfare of growing rabbits as it constrained their movement, especially at earlier ages, conditioned their reactivity towards a new environment, and increased their stress level as measured by physiological indicators. The increase in the stocking density (12 to 16 rabbits/m^2) and of rabbit age (8 wk to pre-slaughter age) did not affect the welfare of rabbits housed in collective pens with a large group size. The weak differences in behaviour and reactivity according to gender do not seem enough to justify separate housing for males and females. Other than the specific objectives of the present study, the simultaneous use of different indicators was key to assessing welfare under different conditions.

Acknowledgements: This work was supported by the Seventh Framework Programme of the European Commission [grant number 600376, CUP C91J13001170006]. The authors wish to thank Dr. Andrea Zuffellato (AIA S.p.A., Italy) for his technical support and assistance.

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