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EFFECT OF ENVIRONMENTAL ENRICHMENT AND GROUP SIZE ON BEHAVIOUR AND LIVE WEIGHT IN GROWING RABBITS

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Abstract: The aim of this research was to study the effects of group size and environmental enrichment on behaviour and growth of 108 hybrid growing rabbits (*Oryctolagus cuniculus*). We compared the behaviour (time budget and reactions to specific behavioural tests: "tonic immobility" and "emergence test") and live weight of growing rabbits housed in cages with a different number of rabbits per cage (2, 3 and 4; same density: 14 rabbits/m^2). Half of the cages were enriched with a wooden stick (cylindrical *Robinia Pseudoacacia,* 20 cm of lenght and 6 cm of diameter) hanging from the ceiling of the cage. The stick and number of animals per cage had no effect on weight gain or on behavioural tests responses. Interaction with the stick was significantly higher at the beginning of the growing period. Principal component analysis performed on the data for the whole period showed significant differences according to the treatments: increasing the number of rabbits per cage and introducing a wooden stick seemed to affect locomotor activity frequency and social interactions. Rabbits housed 3 and 4 per cage showed less lying behaviour and higher locomotor activity and sitting. The larger functional space allowance enabled rabbits to perform more natural behaviours compared to smaller cages (2 rabbits/cage). Environmental enrichment seems to be related to higher allogrooming behaviour frequency, which could indicate a social behaviour related to pheromonal olfactory stimulation and mutual recognition.

Key Words: rabbits, behaviour, environmental enrichment, group size.

INTRODUCTION

Increasing concern towards animal welfare in livestock farming in recent years has led to the study of alternative rabbit housing systems. There are currently no European Directives on housing and management procedures for farmed rabbits, whereas a Scientific Opinion of the EFSA Scientific Panel on Animal Health and Welfare was published in 2005 (EFSA, 2005). The major issues in housing fatteners are the group size and space allowance, as well as environment quality. In Italy, rabbits are usually kept in groups ranging from 2 to 6 rabbits per cage with a space allowance of around 500-700 cm^2/rabbit (Verga *et al.*, 2007). Some authors (Morisse *et al.*, 1999) have indicate that conventional cage housing systems may negatively affect rabbit welfare; they could not perform their species-specific behavioural traits: locomotor activities in particular (running, hopping, rising up, etc.). Moreover, the environment is often barren and sometimes gives rise to abnormal behaviours or stereotypies such as cage bar biting or chewing, aggression or apathy (Jordan *et al*., 2003; Verga *et al.*, 2007).

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To reduce stress due to lack of stimuli and improve rabbit welfare, it is necessary to study appropriate environmental design, considering both space allowance and cage group size (Szendrő *et al.*, 2009). Additionally, rearing environment can be enriched by a variety of devices (Postollec *et al.*, 2006; Newberry, 1995; Lidfors, 1997; Hansen and Berthelsen, 2000) such as inclusion of hiding and resting places in the housing cage (e.g., raised platforms or alternative floors) (Postollec *et al.*, 2008) and the introduction of enrichment objects (wood stick, mirrors etc.) (Princz *et al.*, 2009; Dalle Zotte *et al.*, 2009) or roughage food such as hay (Lidfors, 1997; Berthelsen and Hansen, 1999), grass cubes or gnawing sticks (Love, 1994). The positive effect of object enrichment was mainly recorded in the reduction of aggressive behaviour and in the improvement of animal welfare (Dalle Zotte *et al.*, 2009). Furthermore, a clear preference for object-enriched cages was reported by Princz and colleagues in 2008.

The aim of this research was to investigate the effect of group size and environmental enrichment on commercial hybrid rabbit behaviour and growth performance.

MATERIAL AND METHODS

Animals, housing conditions and object enrichment

This study was carried out in a commercial rabbit farm equipped with an air control temperature system (T: 20±2°C, Light: 16:8 h L:D). One hundred and eight commercial growing hybrid rabbits of both sexes were studied in this trial (sex ratio 1/1). The rabbits were housed according to Table 1 distribution. Each cage was equipped with a feeder and a nipple drinker; feeding (commercial diet: metabolisable energy 2 400 kcal/kg; crude protein 15.0%, automatic distribution) and water were provided *ad libitum*. The environmental enrichment consisted of a wooden stick (*Robinia pseudoacacia,* length: 20 cm×diameter: 6 cm) hanging from the cage ceiling. The rabbits were transferred to the cages at the age of 40 d and randomly assigned to each treatment. Slaughter age was 79 d.

Recorded variables

Performance variables were recorded based on live weight (40-61-79 d of age).

Home cage behaviour: The cages (n=36) area was equipped with 6 aerial video-cameras, time-lapse video-recorders and digital field switchers were used. At 40, 61 and 79 d of age, video-recorded behavioural observations were carried out for a total of 72 h (4 min time lapse recording; 30 min scan sampling) (Martin and Bateson, 1993 modified). From videotapes we distinguished inactive behaviour (sitting and lying) from active behaviours such as ingestion (feeding and

Table 1: Experimental design: groups, number of animals and cages and cage characteristics.

Treatment	No.	Rabbit/cage	Width (cm)	Depth (cm)	Height (cm)	Enrichment (wood-stick)
4E	6	4	68.0	41.5	29.0	yes
4C	6	4	68.0	41.5	29.0	no
3E	6	3	51.0	41.5	29.0	yes
3C	6	3	51.0	41.5	29.0	no
2E	6	2	51.0	28.0	29.0	yes
2C	6	2	51.0	28.0	29.0	no

drinking), comfort (self-body care), social (sniffing, biting, allogrooming), locomotion (walking), interaction with wooden stick (sniffing, gnawing the stick); stereotypies (gnawing the cage bars).

Behavioural test: To evaluate rabbits reactivity and fear responses, 2 behavioural tests were performed at 61 and 79 d of age: N=48, 1 rabbit per cage in 2 and 3 rabbits cages and 2 rabbits per cage in 4 rabbits cages, randomly selected (Hansen *et al.*, 1993; Ferrante *et al.*, 2005).

Emergence test (ET): During the ET, each rabbit was placed in a wooden start box (width ×depth×lenght; 50×50×50 cm), closed by a lid, with a sliding door leading into the closed arena (50×120×50 cm). After 1 min acclimatisation, the sliding door was opened to the arena. The recorded parameters were: No. of escape attempts (the number of times the rabbit put the head or one or more legs outside the box before it emerged) and emergence latency (time for whole body entry into the arena, s, max of 180 s). Long lasting latencies to approach and enter a new arena and a low number of attempts to enter with one or more legs are considered high fearfulness indicators (Miller *et al.*, 2005).

Tonic immobility test (TI): After ET the TI test was performed. The rabbit was laid on its back in a V-shaped wooden device and kept in this position by placing one hand on the animal hind-feet and the other on their ears for 10 s. Hand pressure was gradually lifted till the rabbit stopped moving; if still moving, the induction was considered unsuccessful and another induction period of 10 s started, until movement ceased. If the rabbit stopped and showed tonic immobility, the worker slowly withdrew the hands and a chronometer was activated to measure the TI duration (s, max 180 s); TI ended when the rabbit returned to the upright position. If 3 inductions were unsuccessful, TI duration was scored as 0 and the number of inductions was considered equal to 3. During TI test, the worker always stood close to the rabbit to be seen by the animal.

Statistical analysis

Statistical analysis was carried out using SAS software (SAS, 2008). The level of significance was set at $P<0.05$.

Effects of housing conditions on rabbit weight were calculated using the GLM procedure of SAS (2008) with group size and cage enrichment as source of variation. A principal component analysis (PCA, SAS, 2008) was performed on the whole dataset of behavioural variables. Emergence test results (latency) were fitted with a univariate linear model for repeated measures, wooden stick and number of rabbits being the covariates, while "number of attempts to leave the box" was fitted with a Poisson linear model for repeated measures using the GENMOD procedure from SAS with logarithmic link function and the same covariates. Effects on tonic immobility time were fitted using the GLM procedure of SAS (2008) with a factorial univariate linear model for repeated measures, group size and object enrichment being the covariates. Effects on "number of inductions" were evaluated fitting a Poisson linear model for repeated measures using the GENMOD procedure of SAS, with logarithmic link function, having wood stick and number of rabbits as covariates (SAS, 2008).

RESULTS AND DISCUSSION

No significant effect of object enrichment and group size was observed on animal live weight (Table 2). The 4C treatment showed the highest average live weight values at the 3 ages of recording ($P>0.05$). These results support the findings of previous studies showing that environmental enrichment and housing have a low effect on productive performance and meat quality in rabbits (Szendro *et al.*, 2009; Verga *et al.*, 2004). Univariate analysis of behavioural

Table 2: Effect of cage enrichment and group size on live weight. The least squares means (LSM) and standard errors (SE) are reported. (P>0.05).

		Control		Enriched	
Age (d)	Rabbit/Cage (No.)	LSM (g)	SE (g)	LSM (g)	SE (g)
40	2	1116	97	1043	130
	3	1097	97	1052	130
	4	1161	97	1043	130
61	2	2097	232	2087	174
	3	2123	290	2130	174
	4	2258	142	2087	174
79	2	2813	200	2652	304
	3	2452	355	2765	174
	4	2839	284	2696	174

variables showed that stick interactions are more frequent (P<0.05) during the first period, perhaps indicating a habituation process. Johnson *et al.* (2003) did not find any difference in behavioural or hematologic parameters between rabbits in enriched cages and the control group. The rabbits were individually housed in cage with an environmental enrichment device: stainless-steel rabbit rattles on spring clips. These results could be due to habituation to objects, which reduces the interaction with them over time; it would be possible to rotate the different enrichment objects to achieve the expected effect (Johnson *et al.*, 2003). Various authors (Johnson *et al.*, 2003; Jordan *et al.*, 2003; Luzi *et al.*, 2003; Verga *et al.*, 2004) agree that in enriched cages the frequency of abnormal behaviours of rabbits is lower and gnawing the stick reduces the aggressive forms (Princz *et al.*, 2009, 2008, 2007; Szendrő, 2009).

The effects of cage enrichment and group size on rabbit behaviour are reported in Table 3. No significant differences within the treatments were recorded.

Table 3: Effect of group size (2,3 and 4 rabbits/cage) and cage enrichment (C, control; E, enriched) on rabbit behaviour. Values are mean percentage from scan sampling (40-61-79 d of age; P>0.05). Interaction rabbit: between rabbits interaction activity. Physical contact, smelling etc.

Treatment	2C	2E	3C	3E	4C	4E
Behaviour (%)						
Drink	0.58	0.13	0.45	0.29	0.57	0.35
Eat	47.24	47.44	61.77	60.70	55.50	53.90
Locomotor	10.99	10.35	11.47	11.31	11.60	11.50
Sitting	17.52	16.16	7.10	6.83	13.49	12.98
Lying	1.13	1.09	0.76	0.36	0.49	0.60
Self grooming	14.49	11.49	12.36	10.38	11.17	10.96
Allogrooming	2.26	1.72	2.06	1.46	2.31	2.21
Interaction Rabbit	5.79	2.14	4.03	3.04	4.87	3.67
Interaction Object	0.00	9.48	0.00	5.63	0.00	3.83

Table 4: Eigenvalues (first 3 principal components) of behavioural test correlation.

Principal component	Eigenvalue	Variance (%)	Cumulative (%)
PC1	1.31	14.6	14.6
PC2	1.27	14.1	28.7
PC3	1.07	11.8	40.5

PCA analysis of the recorded behaviours showed that the first 3 principal components explain about 40% of the total variance (Table 4). The first principal component (14.6% of total variance) is positively related to lying and negatively related to sitting and movement. This component discriminates among the number of rabbits per cage and showed that an increased number of rabbits is positively related to these behaviours (Figure 1 and Table 5). The second principal component (14.1% of total variance) is negatively related to lying and "sitting" and positively related to movement. This component discriminates among numbers of rabbits per cage (Figure 1 and Table 5) showing that an increased number of rabbits is positively related to sitting and movement. These results could be related to the higher possibility of movement due to the higher functional space allowance in cages housing 3 or 4 rabbits compared to those housing 2 rabbits. In 2C and 2E cages, the functional space does not allow rabbits to express many locomotor behaviours. In small cages, few body movement activities are seen, but increased rest and feed behaviours have been observed (EFSA, 2005). In this research, the higher functional space allowance in 3C, 3E, 4C and 4E cages permitted a freer movement compared to those housed in bicellular cages. The third principal component (11.9% of total variance) counterpoises allogrooming to the other social interactions (Figure 2). It was related to different types of social activity and showed that the presence of a wooden stick was positively related to allogrooming while the absence of the stick matched with an increased number of the other social interactions, although no real aggressive behaviour was observed.

Environmental enrichment may affect social behaviour, increasing allogrooming which could be related to pheromonal olfactory stimulation. The analysis showed no effects of treatment ($P>0.05$) on the ET and TI test responses. The latency in the emergence test varied from 68-87.6 s, while the duration of immobility tonic ranged from 33.7-60.7 s.

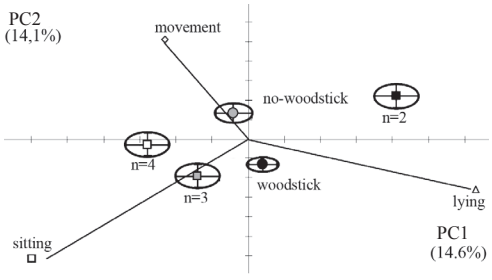

Figure 1: Prinpal components 1 and 2 scan plot (PC1×PC2). Rabbit/cage: n=2, n=3, n=4. Enrichment: woodstick, no-woodstick. Behaviour: lying, sitting, movement.

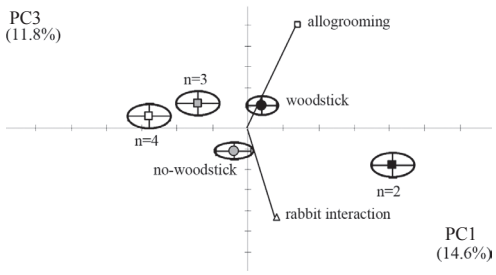

Figure 2: Principal components 1 and 3 scan plot (PC1×PC3). Rabbit/cage: n=2, n=3, n=4. Enrichment: woodstick, no-woodstick. Social activity: allogrooming, rabbit interaction.

Table 5: Eigenvector of correlation matrix of behavioural variables and least square estimates of group scores.

Principal component	PC1	PC2	PC3
Behaviour			
Sitting	–0.61	–0.66	0.14
Movement	–0.25	0.51	–0.04
Lying	0.64	–0.26	–0.25
Allogrooming	0.15	–0.089	0.5
Rabbit interactions	0.079	–0.0025	–0.43
Least square estimates			
2 rabbits/cage	0.41	0.23	–0.18
3 rabbits/cage	–0.28	–0.036	0.065
4 rabbits/cage	–0.12	–0.2	0.12
control cage	–0.049	0.12	–0.12
enriched cage	0.049	–0.12	0.12

CONCLUSION

We can conclude that space allowance is a basic factor in normal behaviour expression in fattening rabbits; moreover, interaction with an object is an important stimulus in rabbit exploratory behaviour expression, particularly in small sized groups. Research works should be addressed to clarify the real meaning of the different social interactions in growing rabbits according to functional space, slaughtering age and environmental enrichment.

REFERENCES

Berthelsen H., Hansen L.T. 1999. The effect of hay on the behaviour of caged rabbit (*Oryctolagus Cuniculus*). *Anim. Welfare, 8: 149-157.*

Dalle Zotte, Princz Z., Metzger S., Szabo A., Radnai I., Biro-Nemeth E., Orova Z., Szendro Z. 2009. Response of fattening rabbits reared under different housing conditions. 2. Carcass and meat quality. *Livestock Sci., 122: 1, 39-47. doi: 0.1016/j. livsci.2008.07.021*

EFSA (European Food Safety Authority) 2005. The impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits. *EFSA Journal, 267: 137.*

Ferrante V., Marelli S., Pignattelli P., Baroli B., Cavalchini L. G. 2005. Performance and reactivity in three Italian chicken breeds for organic production. *Anim. Sci. Pap. Rep. 23 (Suppl.): 223-229.*

Hansen I., Braastad B. O., Storbraten J., Tofastrud M. 1993. Differences in fearfulness indicated by tonic immobility between lying hens in aviaries and in cages. *Anim. Welfare, 2: 105-112.*

Hansen L.T., Berthelsen H. 2000. The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*). *Appl. Anim. Behav. Sci., 68, 163-178. doi: 10.1016/S0168-1591(00)00093-9*

Johnson C.A., Pallozzi W.A., Geiger L. 2003. The effect of an environmental enrichment device on individually caged rabbits in a safety assessment facility. *Contemp. Top. Lab. Anim. 42: 27-30.*

Jordan D., Štuhec I., Pečlin G., Gorjanc G. 2003. The influence of environmental enrichment on the behaviour of growing rabbits housed in individual wire cages. *In Proc.: 13. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere, Celle. 119-126.*

Lidfors L. 1997. Behavioural effects of environmental enrichment for individually caged rabbits. *Appl. Anim. Behav. Sci., 52, 157-169. doi: 10.1016/S0168-1591(96)01141-0*

Love J.A. 1994. Group housing: Meeting the physical and social needs of the laboratory rabbit. *Lab. Anim. Sci., 44: 5-11.*

Luzi F., Ferrante V., Heinzl E., Zucca D., Verga M., Bianchi M., Cavani C., Petracci M. 2003. Effect of enviromental enrichment and group size on performance and carcass traits in rabbits. *In Proc: 54th Annual Meeting of the EAAP, Rome, 203.*

Martin P., Bateson P. 1993. Measuring behaviour: an introductory guide. *2nd ed. Cambridge University Press, Cambridge, UK*

Morisse J.P., Boilletot E., Martrenchar A. 1999. Preference testing in intensively kept meat production rabbits for straw on wire grid floor. *Appl. Anim. Behav. Sci., 64: 71–80. doi: 10.1016/S0168-1591(99)00023-4*

Newberry R.C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. *Appl. Anim. Behav. Sci., 44: 229-243. doi: 10.1016/0168-1591(95)00616- Z*

Postollec G., Boilletot E., Maurice R., Michel V. 2006. The effect of housing system on the behaviour and growth parameters of fattening rabbits. *Anim. Welfare, 15: 105-111.*

Postollec G., Boilletot E., Maurice R., Michel V. 2008. The effect of pen size and an enrichment structure (elevated platform) on the performances and the behaviour of fattening rabbits. *Anim. Welfare 17: 53-59.*

Princz Z., Orova Z., Nagy I., Jordan D., Štuhec I., Luzi F., Verga M., Szendrő Zs. 2007. Application of gnawing stick in rabbit housing. *World Rabbit Sci. 15: 29-36. doi: 10.4995/wrs.2007.607*

Princz Z., Dalle Zotte A., Radnai I., Biro-Németh I., Matics Zs., Gerencsér Zs., Nagy I., Szendro Zs. 2008. Behaviour of growing rabbits under various housing conditions. *Appl. Anim. Behav. Sci. 111: 342-356. doi: 10.1016/j.applanim.2007.06.013*

Princz Z., Dalle Zotte A., Metzger Sz., Radnai I., Biró-Németh E., Orova Z., Szendro Zs. 2009. Response of fattening rabbits reared under different housing conditions. 1. Live performance and health status. *Livest. Sci., 121: 86-91. doi: 10.1016/j.livsci.2008.05.018*

SAS Institute Inc. 2008. *SAS/STAT® 9.2 User's Guide. Cary, NC, USA*

Szendrő Zs., Princz Z., Romvari R., Locsmandi L., Szabo A., Bazar Gy., Radnai I., Biro-Nemeth E., Matics Zs., Nagy I. 2009. Effect of group size and stocking density on productive, carcass, meat quality and aggression traits of growing rabbits. *World Rabbit Sci. 17: 153-162*. doi: 10.4995/wrs.2009.655

Verga M., Zingarelli I., Heinzl E., Ferrante V., Martino P.A., Luzi F. 2004. Effect of housing and environmental enrichment on performance and behaviour in fattening rabbits. *In Proc.: 8th World Rabbit Congress, 7-10 September, 2004. Puebla, Mexico.1283-1288.*

Verga M., Luzi F., Carenzi C. 2007. Effects of husbandry systems on physiology and behaviour of farmed and laboratory rabbits. *Horm. Behav., 52: 122-129. doi: 10.1016/j.yhbeh.2007.03.024*