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Effect of environmental enrichment and composition of the social group on the behavior, welfare, and relative brain weight of growing rabbits

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ABSTRACT

The objective of the study was to investigate if environmental enrichment and the composition of the social group would affect the behavior and relative brain weight of growing rabbits. Rabbits (72 males and 72 females) were assigned to cages with or without enrichment and one of three social groups (males, females, or mixed-gender). Two eucalyptus sticks suspended from the cage ceiling were provided in the enriched cages based on the results of a preliminary trial conducted on growing rabbits (n = 48) showing that pieces of wood (*Eucalyptus sp, Pinus sp*) or bamboo (Dendrocalamus giganteus) were preferred over PVC pipe ($P < 0.05$). Rabbits were exposed to the experimental conditions between six and 11 weeks of age. Behavioral activities were video recorded for 24 h, at 7, 10 and 11 weeks of age. Growth performance was recorded from 42 to 77 days of age, whereas skin wounds and brain weight were recorded at 77 days. At 70 days of age, rabbits in non-enriched cages showed a higher proportion of self-grooming ($P = 0.012$) than those in enriched cages. Enrichment decreased the number of social interactions among rabbits ($P = 0.012$), but increased aggressive behavior ($P = 0.007$). The number of animals showing skin wounds on day 77 was lower ($P = 0.006$) in enriched than in non-enriched cages. The incidence of social interactions was higher ($P < 0.05$) and of stereotypes was lower ($P < 0.05$) in mixed-gender groups than in same-sex groups. Female groups showed the lowest incidence of aggressive behavior ($P < 0.05$). The number of individuals with skin injuries was higher in mixed-gender groups ($P < 0.05$) than in female groups; male groups were intermediate. Growth performance was unaffected by enrichment or by the composition of social group. Males in enriched cages had heavier brains ($P < 0.05$) than those in non-enriched cages. Although aggressive behaviors were more frequent, the number of skin wounds on day 77 was lower in rabbits from enriched cages, suggesting improved welfare. Based on the increased frequency of social interactions and decreased incidence of stereotyped behavior, mixed-gender groups should be housed in collective cages from weaning up to 11 weeks of age. However, if the incidence of skin wounds is considered, only females can be housed in same-sex groups. For males, individual cages should be preferred. Whether environmental enrichment induces morphologic changes in male rabbits' brains should be further investigated.

1. Introduction

Rabbits have been used for decades for intensive lean meat production and also as models in biomedical research such as anti-body production (Hanly et al., 1995; Grosse-Hovest et al., 2004), lipoproteins and atherosclerosis research (Van Zutphen and Fox, 1977; Korstanje, 2000), cardiovascular physiology research (Bõsze and Houdebine, 2006), ophthalmology and vision research (Gwon 2008; Kang and Grossniklaus, 2011), and more recently, in the development of implant dentistry research (Mapara et al., 2012; Stübinger and Dard, 2013).

Both in rabbit farms and in laboratories, animals are frequently kept in barren cages, generating increasing concern with their welfare (Hansen and Berthelsen, 2000). The welfare of growing rabbits kept in cages depends on whether housing is individual,

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in pairs, or in groups, on group size and stocking density, but also on the quality of the environment (Chu et al., 2004; Verga et al., 2007; Trocino et al., 2014). Physiology, health, reproduction, and production may be used to evaluate stress, but the evaluation of the ethogram represents a key welfare indicator in animals kept in intensive conditions. In rabbits, the normal behavioral repertoire includes foraging and social behaviors, but confinement in barren cages reduces locomotion and may lead to altered social behavior and the development of stereotypies (Morisse and Maurice, 1997; Hansen and Berthelsen, 2000; Verga et al., 2007).

Environmental enrichment can be viewed as any modification of the environment that exerts favorable effects on the animals' behavior and ability to cope with captivity, by stimulating their species-specific needs (Newberry, 1995; Olsson and Dahlborn, 2002). It should improve the behavioral repertoire and reduce the frequency of abnormal behavior. For caged rabbits, environmental enrichment may be accomplished by the provision of social companions, by adding structures that can be used as shelters or resting places, such as boxes or elevated platforms, or by providing additional food objects such as straw, alfalfa cubes or wooden sticks to meet the animals' need for chewing (Jordan et al., 2006).

Using straw as cage enrichment had positive effects on rabbit behavior by increasing the frequency of exploratory activities and decreasing stereotypes and self-grooming (Siloto et al., 2008). Straw on the cage floor, however, may favor feces accumulation and represent a sanitary risk. A "u" shaped wooden structure decreased cage manipulation and interactions with conspecifics, probably because it was used as a shield against unwanted social contact (Buijs et al., 2011). The most commonly used enrichment for growing rabbits is the wooden stick. It was found to improve the welfare of growing rabbits through the reduction of body injuries (Princz et al., 2008).

Despite the body of work regarding rabbit behavior under various environmental conditions, few studies have focused on underlying physiological or physical changes. For example, in rodents and other animal species, the addition of environmental stimuli was found to increase brain weight (Diamond et al., 1964) by enhancing cortical thickness and dendritic ramification (Beaulieu and Colonnier, 1987; Rosenzweig and Bennet, 1996), stimulating neurogenesis in the hippocampus (Kempermann et al., 1997), and improving memory in learning tasks (Van Praag et al., 2000). However, none of the structural or functional changes resulting from environmental enrichment has been investigated in rabbits.

In nature, small groups of one to four male and one to nine female rabbits with a clearly defined hierarchy are common (Trocino and Xiccato, 2006). Social rank orders in these groups are established and maintained by intensive fights (Von Holst et al., 2002). Based on the complex social structure of wild rabbits, domestic animals should be raised in groups both in the reproduction and growth phases (Trocino and Xiccato, 2006; Szendrö and Dalle Zotte, 2011). In rabbit farms, however, breeding males are individually caged due to fighting and breeding females due to competition for nest boxes during late pregnancy and lactation. Fattening rabbits, on the other hand, are kept in collective cages, in which group size depends on cage dimensions (Verga et al., 2007). The EFSA (2005) recommends a minimum of 3500 cm^2 available cage floor surface for breeding males and females and at least 625 cm^2 individual surface for fatteners with a maximum of 40 kg live weight/m^2. Nevertheless, this poses a problem when the growing rabbits reach puberty and the risk of aggressive behavior increases, especially among males. The larger and the older the group, the higher the frequency of severe wounds due to fighting (Trocino and Xiccato, 2006). Laboratory rabbits are usually housed in individual cages that are often small and lack any sort of stimuli (Gunn and Morton, 1995; Hansen and Berthelsen, 2000). Space allowances according to the European Council Directive 2010/63/EU are 800 cm^2/rabbit from weaning up to 7 weeks of age and 1200 cm^2/rabbit from 7 to 10 weeks of age. For rabbits over 10 weeks of age, the minimum floor area per animal ranges from 3500 to 5400 cm^2 according to body weight.

Previous studies have investigated the effects of housing system (Trocino et al., 2014), group size, cage size, and stocking density (Chu et al., 2004; Princz et al., 2008; Buijs et al., 2011) on the behavior of growing rabbits. However, the potential effects of changes to the composition of the social group on social interaction, aggression and injuries have been largely neglected in rabbits, except for one study which showed no effect of social group composition on behavior (Szendrö et al., 2012).

The objective of the present study was to investigate if environmental enrichment and the composition of social group would affect the behavior, welfare and relative brain weight of growing rabbits. The hypothesis was that an enriched environment would improve the welfare of growing rabbits by stimulating motor activity, investigatory and social behaviors and ultimately influencing brain development. But this response could be dependent on the composition of the social group. To investigate this hypothesis, rabbits were housed with or without enrichment and in one of three social group compositions (males, females, or mixed-gender). Environmental enrichment material was selected based on the results of a preliminary trial conducted on growing rabbits.

2. Material and methods

One hundred and ninety-two rabbits from the Botucatu genetic group (Bianospino et al., 2006) were used in two experiments conducted at the UNESP Rabbit Production Unit, located in Botucatu, SP, Brazil. At weaning, at 35 days of age, animals had three digit numbers tattooed in their ears for identification and were housed in an east–west oriented building equipped with commercial wire cages (0.80 × 0.60 × 0.45 m, *Gaiolas Londrina*, Brazil). The building had plastic adjustable curtains used to control ventilation. Illumination was natural, but four black light bulbs were used during the dark period for behavioral observation or video recording. Local (22°51′S, 48°26′W) natural photoperiod is approximately 11.5 h in April, decreasing to 10.5 h in June (June 21 is the winter solstice) and increasing to 13.5 h in December (December 21 is the summer solstice). Drinking water and a commercial pelleted feed (*Agromix Nutrição Animal*, Jaboticabal, Brazil) were provided *ad libitum*. All the animal procedures were approved under protocol number 011/2010 by the Faculdade de Medicina Veterinária e Zootecnia, UNESP, IACUC on February 10, 2010.

2.1. Preference test

A preliminary trial was conducted in April to test for the growing rabbits' preference for potential cage enrichment materials. The choice of materials was based on availability and cost, thus pieces of wood (0.15 × 0.03 × 0.03 m) (*Eucalyptus sp*, *Pinus sp*), bamboo (*Dendrocalamus giganteus*), and PVC pipe (*Tigre*, Brazil) were used. The last two were 0.15 m long and 0.03 m in diameter. Forty-eight weaned male and female rabbits were allocated six per cage, in a completely randomized design with four treatments (enrichment materials). In each cage at least two rabbits were from the same sex. Two different enrichment pieces were suspended 0.43 m apart with wire from the cage ceiling and their height was adjusted weekly according to the animals' size, from 0.20 to 0.30 m above the cage floor (Fig. 1). The number of interactions with the enrichment materials (Table 1) was counted over four one-hour periods