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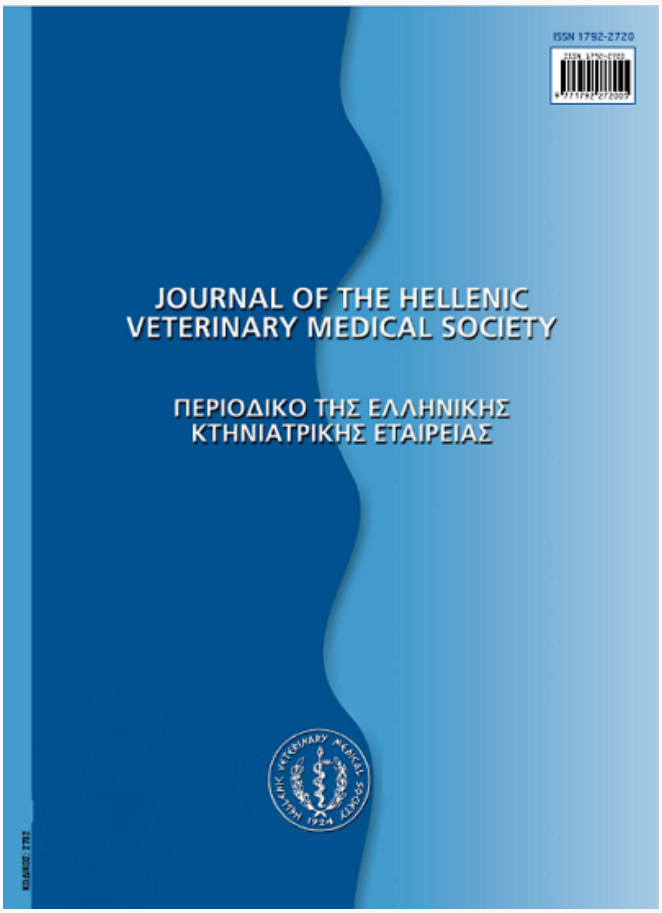

Age - related behaviour on individually caged rabbits

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Age – related behaviour on individually caged rabbits

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Μελέτη της συμπεριφοράς ατομικά στεγαζόμενων κονίκλων σε σχέση με την ηλικία

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ABSTRACT. Housing conditions and environmental enrichment of individually caged laboratory rabbits is of great importance for the welfare of the animals and the quality of the experimental results. In order to improve the design of existing environmental enrichment programs for laboratory rabbits, considerable knowledge of the behavioural needs of this species is necessary. Taking this into consideration, the aim of this study was to monitor and analyze the behaviour of juvenile and young adult rabbits in order to establish whether there are any age-dependent differences in grooming, rearing, sniffing, eating, drinking and gnawing. 12 NZW rabbits were divided into two groups: group A consisted of six 6-month-old rabbits (young adults) and group B consisted of six 2-month-old rabbits (juvenile). All animals were already housed for more than twenty days under the same conditions in the animal facility. Both groups of rabbits were video-recorded between 06:00h – 18:00h for four consecutive days. The frequency of each behaviour was determined and compared in the two groups of rabbits from the video recordings. The frequencies of grooming, eating and gnawing in the young rabbits were significantly greater than those in the older rabbits ($p<0.05$). No statistical differences were found between the two groups for rearing, sniffing and drinking. From these results, we concluded that even small age differences should be taken into account when designing an environmental enrichment program for individually caged rabbits.

Keywords: rabbit welfare, environmental enrichment, age, behaviour, individual caging

ΠΕΡΙΛΗΨΗ. Οι συνθήκες στέγασης και ο εμπλουτισμός του περιβάλλοντος των κονίκλων που στεγάζονται σε ατομικούς κλωβούς αποτελούν θέματα ιδιαίτερης σημασίας τόσο για τη διασφάλιση της ευζωίας των στεγαζόμενων ζώων όσο και για την

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ποιότητα των ερευνητικών αποτελεσμάτων. Στην προσπάθεια για βελτίωση του περιβάλλοντος στέγασης των ζώων αυτών είναι απαραίτητο να γνωρίζουμε σε βάθος τόσο τη συμπεριφορά που τα ζώα αυτά εκδηλώνουν όταν είναι ελεύθερα στο φυσικό τους περιβάλλον όσο και τις συμπεριφορικές τους ανάγκες όταν αυτά βρίσκονται σε αιχμαλωσία. Στα πλαίσια αυτά σκοπός της συγκεκριμένης μελέτης ήταν να παρακολουθήσει, να καταγράψει και στη συνέχεια να αναλύσει τη συμπεριφορά κονίκλων διαφορετικής ηλικίας προκειμένου να προσδιορίσει τις τυχόν συμπεριφορικές διαφορές λόγω ηλικίας. Επιλέχτηκε να μελετηθούν έξι χαρακτηριστικές και συγκεκριμένες κινήσεις που τα ζώα πραγματοποιούν καθημερινά. Οι κινήσεις αυτές ήταν ο καλλωπισμός (grooming), η όσφρηση (sniffing), το τέντωμα (rearing), η κατανάλωση τροφής (eating), η κατανάλωση νερού (drinking) και το ροκάνισμα (gnawing). Χρησιμοποιήθηκαν 12 αρσενικοί κόνικλοι φυλής New Zealand White οι οποίοι χωρίστηκαν σε δύο ομάδες των έξι ατόμων. Η ομάδα Α αποτελείται από 6 ζώα ηλικίας 6 μηνών και η ομάδα Β από 6 ζώα ηλικίας 2 μηνών. Όλα τα ζώα στεγάζονταν ήδη στις εγκαταστάσεις εκτροφής για περισσότερο από 20 ημέρες. Η παρακολούθηση των δύο ομάδων έγινε μέσω κάμερας προσαρμοσμένης σε ηλεκτρονικό υπολογιστή μεταξύ 06:00 – 18:00 για τέσσερις συνεχόμενες ημέρες. Η συχνότητα εκδήλωσης της κάθε συμπεριφοράς καταγράφηκε και συγκρίθηκε μεταξύ των δύο ομάδων. Η συχνότητα εμφάνισης του καλλωπισμού, της κατανάλωσης τροφής και του ροκανίσματος στα νεαρότερης ηλικίας ζώα της ομάδας Β ήταν σημαντικά μεγαλύτερη από αυτή των μεγαλύτερων ζώων της ομάδας Α ($p<0.05$). Σε ό,τι αφορά τις υπόλοιπες κινήσεις όπως το τέντωμα, την όσφρηση και την κατανάλωση νερού δε βρέθηκαν στατιστικά σημαντικές διαφορές μεταξύ των δύο ομάδων. Συμπερασματικά, όταν οι κόνικλοι θα πρέπει να στεγαστούν σε ατομικούς κλωβούς, ο εμπλουτισμός του περιβάλλοντός τους θα πρέπει να λαμβάνεται σοβαρά υπόψιν. Κατά το σχεδιασμό ενός προγράμματος εμπλουτισμού περιβάλλοντος θα πρέπει να λαμβάνεται σοβαρά υπόψιν η ηλικία των ζώων που πρόκειται να στεγαστούν. Ακόμα και μικρές ηλικιακές διαφορές μπορούν να επηρεάζουν την εκδήλωση διαφόρων συμπεριφορών.

Λέξεις ευρετηρίασης: ευζωία κονίκλου, εμπλουτισμός περιβάλλοντος, ηλικία, συμπεριφορά, ατομική στέγαση

1. Introduction

The New Zealand White (NZW) rabbit (*Oryctolagus cuniculus*) is a commonly used laboratory animal. A widespread practice is to house rabbits individually in plastic or stainless steel cages when these animals are used for antibody production. Single caging isolates rabbits from physical or social contact with other animals and restricts their freedom to carry out natural behaviour like allogrooming, sniffing each other, rearing and sitting up on the hind legs, digging for the purpose of nest-building and foraging (Podberscek et al. 1991, Stauffacher 1992). The inability to perform certain behaviours is thought to lead to intentional movements, or inappropriate or abnormal behaviours, like under- or over- grooming and eating, is thought to lead to hairballs and intestinal stasis, weight loss and obesity. (Dantzer 1986, Jackson 1991, Morton et al. 1993, Gunn 1994). Such behaviours indicate maladaptation and provide clear evidence of a need for some environmental improvement.

Commonly used methods, which could improve the cage environment, are the design and the available space of the cage itself, as well as the enrichment of the place with objects that encourage species-specific behaviour and enhancement of the animals' well-being (Morton et al. 1993, Batchelor 1991, Gribb 1993). According to the new European Directive 2010/63, rabbit cage dimensions were increased, while environmental enrichment is strongly recommended by stating that: *establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species* (European Union 2010).

The aim of an environmental enrichment program is to help the animal to express its behavioural needs in a more appropriate way. As these needs may vary depending on the age, the sex and the social rank, environmental enrichment must consider the needs of the animal to avoid creating more problems.

In this sense, the aim of the present study was to investigate the age-related behaviour between juvenile and young adult rabbits in order to understand their needs better. This knowledge will be helpful for the design and environmental enrichment caging schemes which could be implemented in the everyday practice.

2. Materials and methods

The study was performed in the animal facility of the Center for Experimental Surgery of the Biomedical Research Foundation of the Academy of Athens. The facility is registered as a "breeding" and "experimental" facility, according to the Greek Presidential Decree 160/91, which harmonizes national legislation with the European Directive 2010/63 on the protection of

animals used for scientific purposes (European Union 2010).

2.1 Animals

The observations were carried out with twelve male New Zealand White (NZW) rabbits. Animals were divided in two groups. Group A (n=6) consisted of 6 months old young adult rabbits and group B (n=6) of 2 months old juvenile rabbits, weighing about 5.5 and 2.8 kg, respectively. All animals were reported free of *Pasteurella multocida, Salmonella spp, Treponema cuniculi* and *Eimeria spp*.

2.2. Husbandry

All rabbits were housed and cared for in accordance with the European Commission Recommendations for the accommodation and care of animals used for experimental and other scientific purposes (European Commission 2007).

All animals were housed individually, in single cages on stainless-steel rabbit racks (figure 1). The cage dimensions were 670 (W) x 670 (L) x 470 (H) mm (Tecniplast, Italy). Each cage consisted of a perforated plastic floor (no bedding material was included) and walls and metal bars in front. Cage cards containing specific information for each rabbit in order to detect them from each other were attached to the cages. The rabbits were maintained at a 12:12-h light:dark cycle (lights on between 06:00h and 18:00h). Room temperature was approximately 21°C and relative humidity was 50±5%. Rabbits were fed *ad libitum* with a pre-packaged certified fiber rabbit chow (12C, Pezzulo, Italy). All rabbits had unlimited access to water via automatic watering systems.

After a caging period of more than twenty days the rabbits were video recorded for 4 consecutive days.

2.3 Ethogram

For the behaviour observation, the ethogram shown in Table 1 was used (modified from Gunn and Morton 1995, Hansen and Berthelsen 2000, Krohn et al. 1998, Lidfors, 1997).

2.4. Video recording

Video recordings were made over the light cycle and for a period of four consecutive days. For the purpose of the video recordings, we used an msi Star Cam Flip webcam connected to a computer (NB200-13T, Toshiba). As rabbits remain less active during the light period (Gunn and Morton 1995), a special computer software program (Cam Wizard) detecting motion was used.

Table 1. Ethogram used during the behaviour observation

Behaviour movements	Definition
Self-grooming	the rabbit is licking or biting its fur or washing its ears with its forepaws
Rearing	the rabbit is sitting with the front paws lifted from the floor and standing on hind paws with body stretching upwards and front paws resting on the cage wall
Sniffing	the rabbit is sniffing its environment (i.e. walls, floor, bars, hopper and the roof of the cage) or sniffs the air with its nose at the bars of the cage
Eating	the rabbit is eating pellets from the hopper
Drinking	the rabbit is lapping water with its tongue from the nipple of the bottle
Gnawing	the rabbit is biting the bars or the floor

The video records were reviewed to transform the behavioural observations into quantitative data. For each rabbit, the total number of times a particular movement occurred resulted in a frequency value. The means and standard deviation values of the frequency of occurrence per rabbit were plotted for each behaviour in order to detect patterns of behaviour.

The frequency of each behaviour was determined and compared between the two groups from the two video recordings in order to obtain a total activity level.

2.5 Statistical analysis

All data were tested statistically by using Student's two tailed *t-test*. All statistical analysis was performed using Graph Pad Prism 4 (1992-2005, GraphPad Software, Inc, Dennis Radushev). The significance level was set at $p<0.05$.

3. Results

3.1. Grooming

During the observation period, juvenile rabbits expressed grooming behaviour with a significant higher frequency ($p<0.05$) than the young adults (Figure 1). An increased frequency of grooming behaviour seems to be expressed in both groups when lights were turned

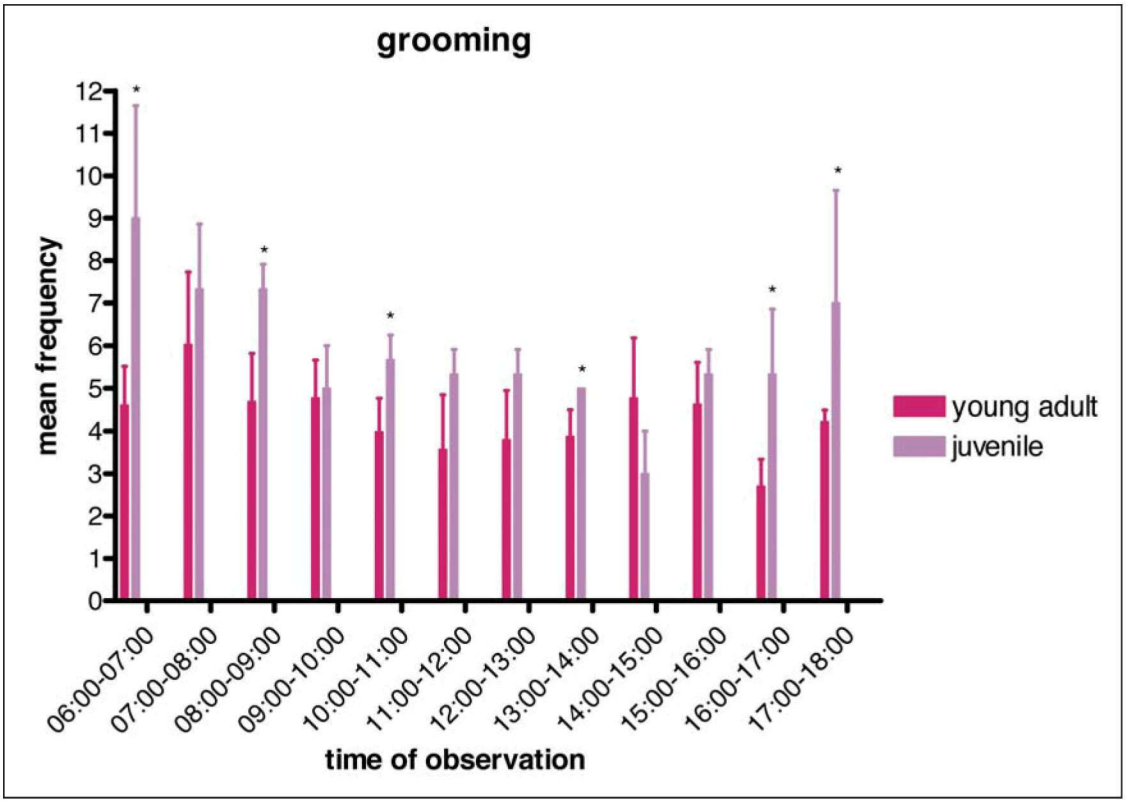

Figure 1. Mean frequency (±SD) of grooming behaviour per group during video recording for the four consecutive days of observation (*) $p<0.05$.

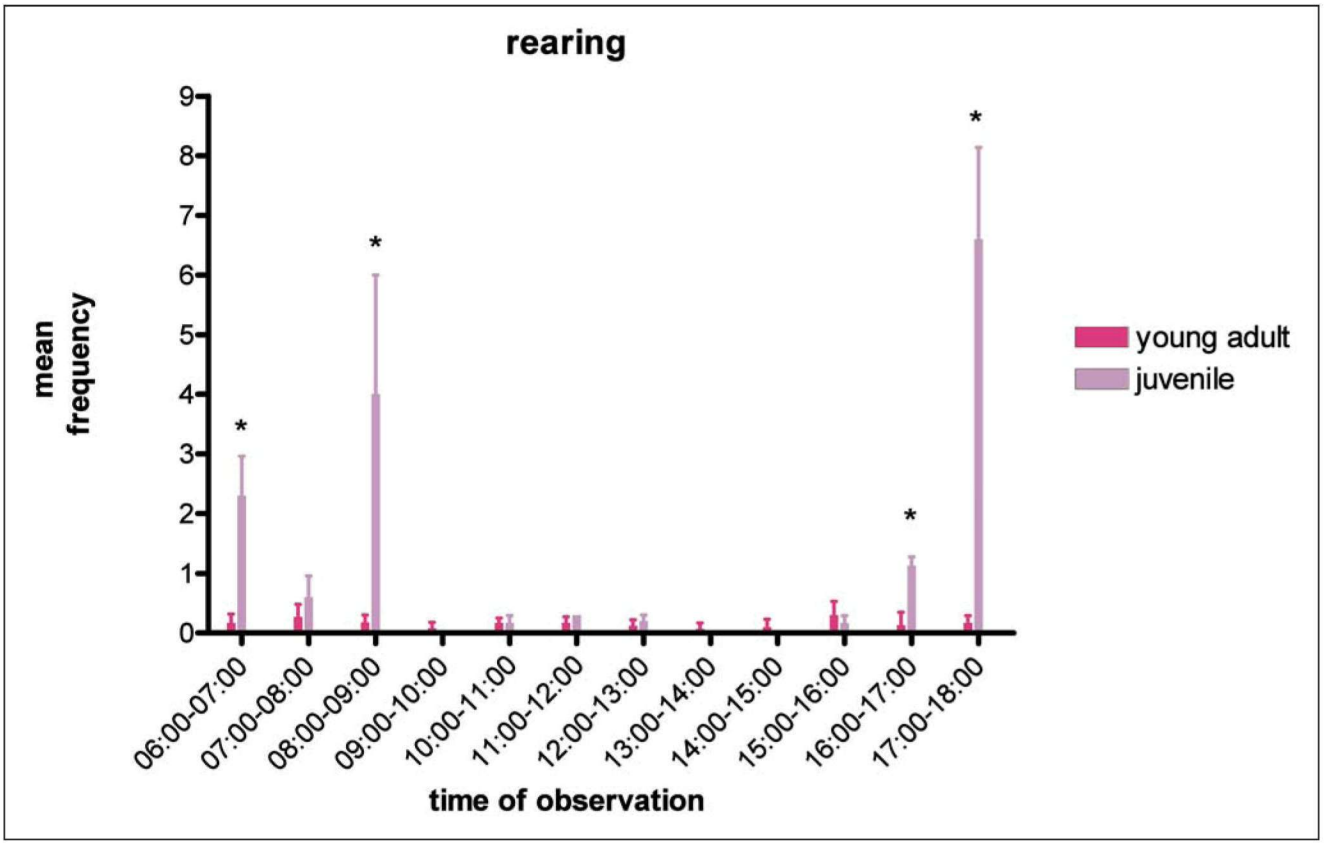

Figure 2. Mean frequency (±SD) of rearing behaviour per group during video recording for the four consecutive days of observation (*) $p<0.05$.

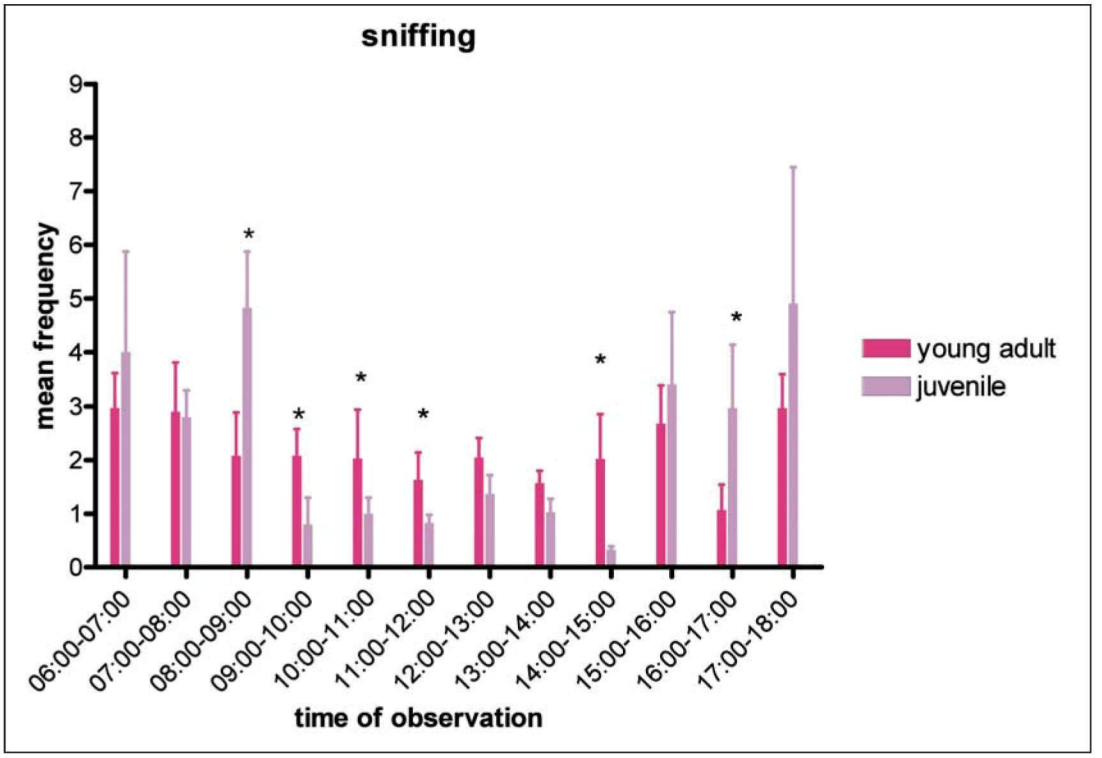

Figure 3. Mean frequency (±SD) of sniffing behaviour per group during video recording for the four consecutive days of observation (*) $p<0.05$.

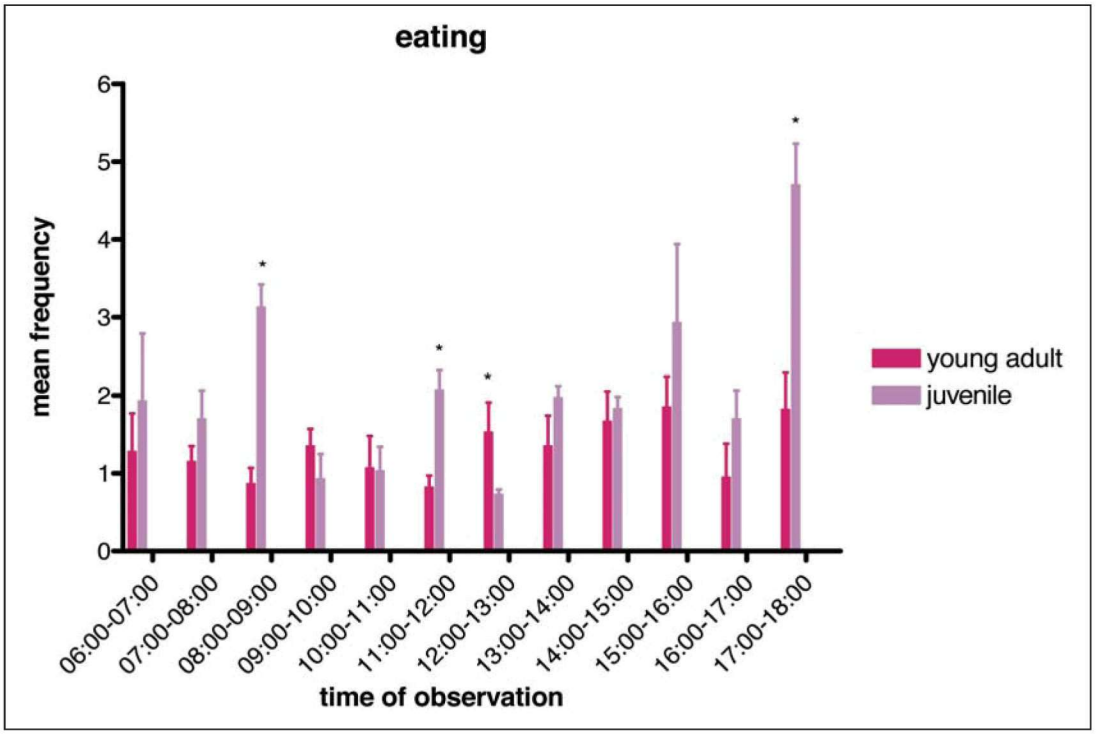

Figure 4. Mean frequency (±SD) of eating behaviour per group during video recording for the four consecutive days of observation (*) $p<0.05$

on and just before they turned off, but this was not statistically significant.

3.2. Rearing

Although the frequency of rearing behaviour doesn't seem to be significantly different when analyzing time periods in total ($p>0.05$), rearing frequency is higher in younger rabbits, especially for the time periods 06:00-07:00, 08:00-09:00 and 16:00-

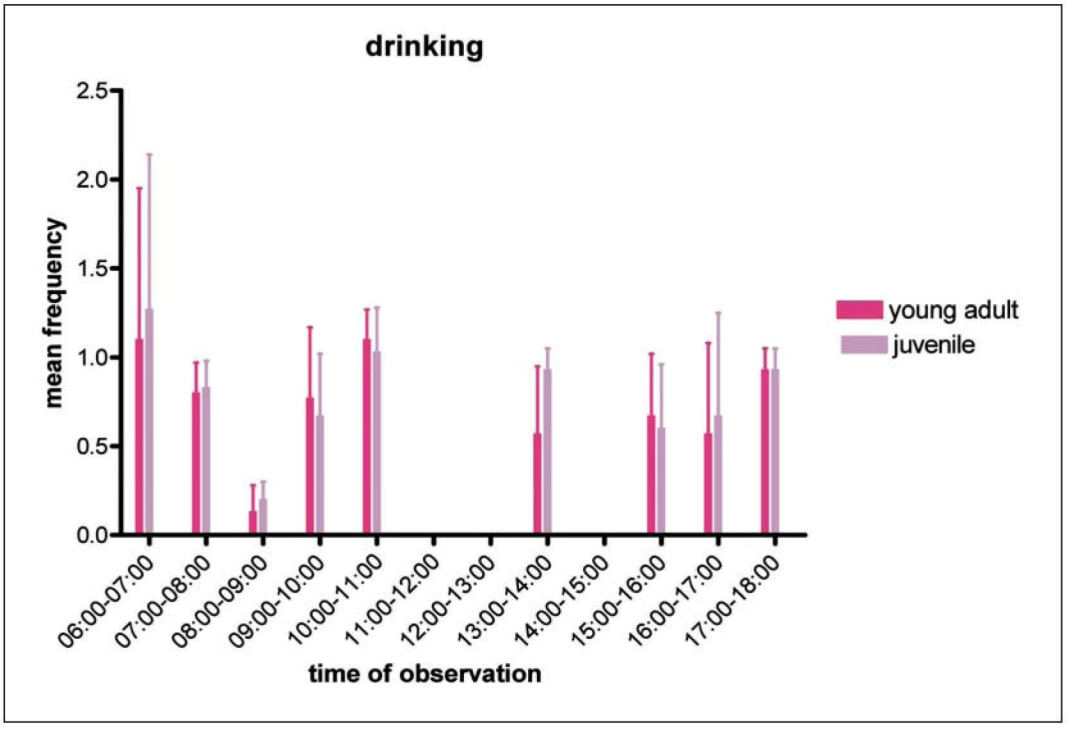

Figure 5. Mean frequency (±SD) of drinking behaviour during the light period (06.00-18.00) per group during video recording for the four consecutive days of observation.

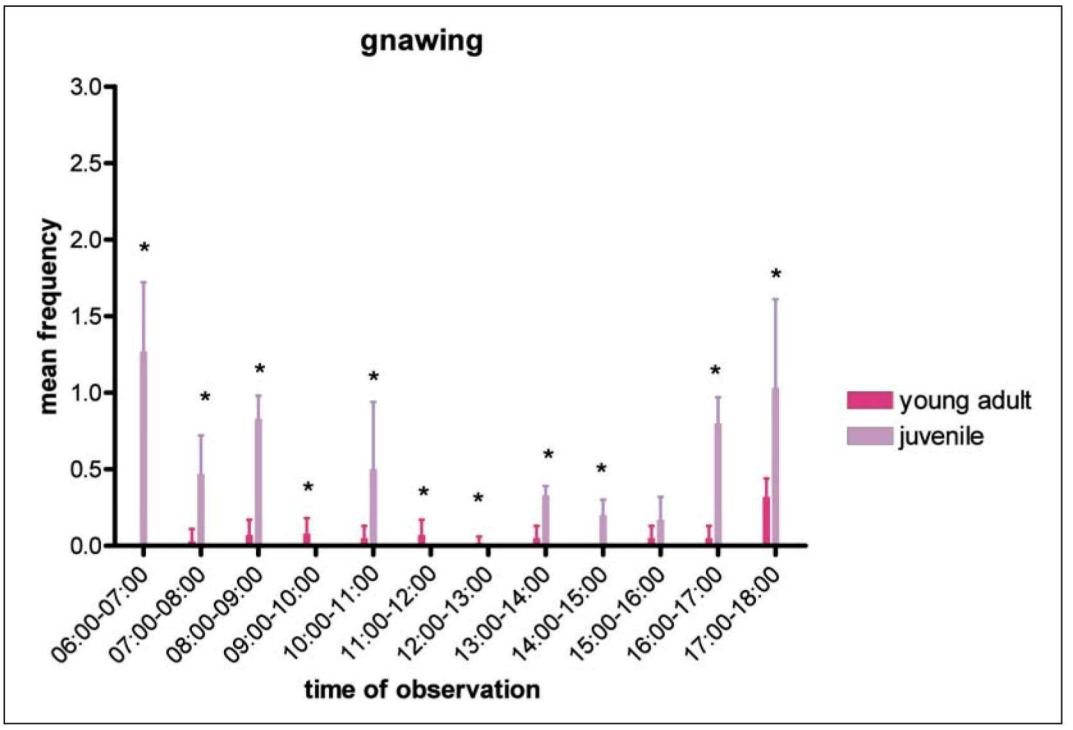

Figure 6. Mean frequency (±SD) of gnawing behaviour per group during video recording for the four consecutive days of observation (*) $p<0.05$.

18:00 for the major part of the day (Figure 2).

3.3. Sniffing

A statistically significant difference was noticed on sniffing frequency for the juvenile rabbits at different time periods (Figure 3). The same tendency of increased activity at the first and the last hours of the day observed in grooming are seen in sniffing, too.

3.4. Eating

Eating frequency, generally, is higher in the juvenile rabbits than in the young adults and the

differences between them are significant ($p<0.05$) at 08:00-09:00, 11:00-13:00, 17:00-18:00 hours. An increased eating behaviour was clearly expressed in juvenile animals at the end of the light cycle (Figure 4).

3.5. Drinking

There is no statistically significant difference between the animals of both groups ($p>0.05$). Juvenile and young adults animals seem to express similar frequencies for water consumption during the same time periods (Figure 5).

3.6. Gnawing

Gnawing frequency is significantly higher for the juvenile rabbits than the young adults ($p<0.05$) during the whole observation period. Furthermore, increased activity was observed during the first and the last hours of light cycle (Figure 6).

Discussion

Although rabbits are social animals and group housing is recommended, there are still cases where rabbits are housed individually in a relatively barren cage environment with restricted or ad libitum amount of diet and water *ad libitum*.

As a result, individual housing in cages is influencing the expression of normal behaviour which, in many cases, could be detrimental for the psychological well-being of the animals (Brooks et al. 1993, Morton 1994). During the last decades, there has been a growing trend of improving caging conditions by using environmental enrichment schemes. Environmental enrichment is, by definition, any modification in the environment of the captive animals that seeks to enhance their physical and physiological well-being, by providing stimuli that meet the animals' species-specific needs (Baumans 2005).

Environmental enrichment is essential in rabbit life for one more reason. Gunn and Morton (1995) indicated that rabbits can stay inactive for most of the 24h. In agreement with this, Mykytowycz and Fullagar in 1973 found that wild rabbits are inactive for 61% of the 24h. This could be interpreted as a reason for not providing rabbits with shelves or more space. However, "being motionless" for quite a long time, cannot be considered as normal behaviour and can be seen as a behavioural sign of increased stress or boredom (Hughes and Duncan 1988). Thus, enrichment of the environment of rabbits is very important to avoid this situation of boredom in order to safeguard their welfare. More information regarding rabbit enrichment highlights pair-housing or the use of food-enriched objects (Harris et al. 2001, Lidfords 1997, Whary et al. 1993).

The choice of a particular environmental enrichment program should begin with a thorough understanding of species-specific behaviour. Considering that age might be a major factor influencing the expression of normal behaviour of the rabbit, the aim of our study was to study the influence of age on the expression of six selected behaviours, self-grooming, rearing, sniffing, eating, drinking and gnawing (Krohn et al. 1999, Gibb 1993). The choice of the age range was based upon our experience that rabbits used for experimental purposes are usually between 2 and 6 months old.

The analysis of video recordings, during the light cycle period, demonstrated that several differences in the rabbits' behaviour were noticed between the two groups. Significantly increased frequencies of self-grooming, eating and gnawing were noticed in juvenile rabbits, while no significant differences could be found in rearing, sniffing and drinking between juvenile and young adult rabbits.

Although we didn't monitor the duration of the behaviours, the frequency of grooming findings in the young adults seem to be similar to those referred to by Hansen and Bethelsen (2000). Grooming frequency in the juvenile animals seemed to be higher. Excessive grooming could be indicative of an under stimulating cage environment (Hansen and Berthelsen, 2000) or social deprivation (Gunn and Morton 1995), disturbance of the animals (Guild and Gunn 1982, Gunn and Morton 1995) or even stereotypic behaviour. No matter what the reason is, age seems to be related to over-expression of such behaviour.

Gnawing of metal bars and plastic floor is characteristic of an abnormal behaviour (Morton 1993, Lidfords 1997) indicating that animals have difficulties in coping with their environment. This abnormal behaviour is clearly expressed more frequently in juvenile animals in the present study and, especially, during the first three hours of the light cycle and two hours before the dark period. Without underestimating the significance of environmental enrichment in all caged rabbits, the over-expression of this abnormal behaviour in the juvenile animals urges the need for additional improvement of the environment. Social enrichment, frequent human

contact, a more complex environment or even food enrichment could improve the housing conditions.

Eating behaviour seems to be expressed more frequently in juvenile animals than in the young adults. Food was provided *ad libitum,* so rabbits could express eating behaviour during the observation period. Juvenile animals expressed an increased frequency of food consumption one hour before the dark period. This could probably be explained by the fact that rabbits in the wild spend most of their time foraging late afternoon or at night (Gidd 1993, Krohn et al. 1998).

In conclusion, when rabbits have to be housed individually in cages, environmental enrichment should be seriously considered. When designing or implementing an environmental enrichment program for individually caged rabbits special concern should be given to their age. Even small age differences of the rabbits may influence the expression of specific behaviours.

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