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Summary

Rabbits should when possible be kept in social groups in pens but as males turn aggressive when sexually mature it is difficult to find an alternative to individual caging. To prevent boredom a rabbit could be given access to an exercise pen. The purpose with this study was to evaluate the use of exercise pens as an environmental enrichment for laboratory rabbits by comparing the behaviour and health of animals that had varying access.

Twentyone male New Zealand White rabbits (4 months – 3 years old) were used. They were housed individually and also used as stud males. They were housed in double stainless steel cages with shelves (10 400 cm^2) in randomized blocks and provided autoclaved hay and aspen wood chew blocks. Three exercise pens (3.65x0.9x0.76 m) of plastic coated steel bar frames were placed in the middle of the animal room on rubber carpets. The pen had a combined shelf/hide, a plastic box filled with wood shavings and hay, a water bottle, a wood chew block and a plastic ball. The rabbits were allocated to three treatment groups: EX1/W were placed in the exercise pens during 1 hour once per week, EX3/W during 1 hour three times per week and controls were kept in their cages with no access to exercise pens. Exercise sessions were carried out during 8 weeks. Behaviour in the cages was observed during some days when there were no exercise sessions and one day while other rabbits were being exercised. Animals were observed during 2 hours and behaviour was recorded instantaneously at two minute intervals. Behaviour in the pens was recorded instantaneously at one minute intervals during 1 hour, and frequency of some behaviour were recorded during 3 min/session. Blood samples were taken in the central ear artery before and after exercise during weeks -1, 1, 4 and 8. The samples were analysed for corticosterone levels and general health parameters. Body weights were recorded once a week during the entire study period. A linear statistical model was fitted to the behavioural data and a pair wise t-test was used for the corticosterone.

Moving was the most common behaviour in the pens, and higher than in the cages. Lying was most common in the cages, whereas in the pens it was shown significantly less. Sitting was more common in pens than in cages, whereas grooming was more common in cages than in pens. Eating did not differ between pens and cages. Hiding occurred only in the pens but at only 0.5-1% of recordings, and mainly during the first exercise session. Young rabbits had a significantly higher number of recordings for leaping and rearing in their pens. Older rabbits instead had a significantly higher percentage of grooming. In the cages young rabbits had a significantly higher number of recordings for eating. Older rabbits instead had a significantly higher percentage of lying. The behaviour performed in cages seemed to be affected by other rabbits being exercised. Corticosterone was elevated after exercise the first week compared to the week before exercise in EX1/W, but not during week 4 and 8. No adverse health effects could be detected in the general health parameters. Rabbits exercised lost some weight during the exericse period compared to controls.

In conclusion animals were more active in the pens and there seemed to be no differences between rabbits exercised once or three times per week. Rabbits in cages also seemed more active while others were being exercised. Signs of improvement in their physical health could be seen as they lost weight and no signs of stress could be measured after the first exercise.

Sammanfattning

Kaniner bör hållas i grupper men då hanar blir aggressiva vid könsmognad är det svårt att finna ett alternativ till att hålla dem individuellt i burar. Rasthagar skulle kunna användas för att berika dessa kaniners miljö. Syftet med denna studie var att utvärdera användandet av hagar för rastning som en berikning för kaniner som används i försök. Detta gjordes genom att jämföra beteende och olika hälsoparametrar hos djur med varierande tillgång.

Tjugoen hankaniner av rasen New Zealand White (4 månader – 3 år gamla) användes. Djuren hölls individuellt och användes som avelshanar. De hölls i dubbla burar i rostfritt stål med hyllor (10 400 cm^2) i randomiserade block och de hade tillgång till autoklaverat hö samt gnagpinnar av asp. I djurrummet byggdes tre rasthagar (3.65x0.9x0.76 m) av ”plastade metallgaller” på gummimattor. I varje hage fanns en kombinerad hylla/gömsle, en plastlåda med kutterspån och hö, en vattenflaska, en gnagpinne och en plastboll. Kaninerna delades in i tre behandlingsgrupper: EX1/W rastades under 1 timme per vecka, EX3/W under 3 timmar per vecka och kontrolldjuren hölls i sina burar. Rastningarna pågick under 8 veckor. Beteende i burarna observerades under vissa dagar när inga djur rastades och under en dag medan andra djur rastades. Djuren observerades under 2 timmar och beteenden registrerades momentant i intervall om två minuter. Beteenden i hagar registrerades momentant i intervall om en minut under en timme och frekvensen av vissa beteenden registrerades under 3 minuter per rastning. Blodprover togs i den centrala öronartären före och efter rastning under veckorna -1, 1, 4 och 8. I proverna analyserades kortikosteronnivåer och generella hälsoparametrar. Kroppsvikter registrerades en gång per vecka under hela studieperioden. En linjär statistisk modell användes för att analysera beteendedata och ett parvis t-test för analys av kortikosteronnivåer.

Rörelse var det vanligaste beteendet i hagarna, och högre än i burarna. Liggande var vanligast i burarna, medan det i hagarna visades betydligt mer sällan. Sittande var vanligare i hagarna än i burarna, medan putsande var vanligare i burarna än i hagarna. Andelen ätande skilde inte mellan hagarna och burarna. Kaninerna försökte sällan att gömma sig i hagarna och det utgjorde bara 0.5-1% av observationerna vilka främst registrerade under den första rastningen. Unga kaniner hade fler registreringar av “glädjeskutt” och att stå på bakbenen i hagarna. Äldre kaniner hade istället fler registreringar av putsande. I burarna hade unga kaniner fler registreringar av ätande och äldre kaniner istället fler av liggande. Beteende i burarna verkade påverkas av att andra kaniner rastades. Kortikosteronnivåerna var förhöjda efter rastning under den första veckan för EX1/W, men inte under vecka 4 och 8. Inga skadliga hälsoeffekter kunde upptäckas i de generella hälsoparametrarna. Kaniner som rastades gick ner i vikt jämfört med kontrolldjuren under studieperioden.

Sammanfattningsvis så var djuren mer aktiva i hagarna och det verkade inte vara några skillnader mellan kaniner som rastades en eller tre gånger per vecka. Kaninerna verkade också bli mer aktiva i sina burar när andra rastades. Tecken på en förbättring av deras fysiska hälsa kunde ses i och med att de gick ner i vikt och inga tecken på stress kunde ses efter den första rastningen.

Introduction

Background

Wild rabbits

Our domesticated rabbits are ancestors of the European wild rabbit (Harcourt-Brown, 2002). The social behaviour of rabbits has been described as very complex and their behaviour has not changed significantly through domestication. In fact behaviour is known to be genetically conservative (Vastrade, 1986; Lehmann, 1991). Studies of domestic rabbits in a near to nature environment have shown that they have the same social behaviour as their wild relatives (Vastrade, 1986; Lehmann, 1991). Wild rabbits live in social groups consisting of one or more males and one or several females (Lehmann, 1991, Lidfors & Edström 2010). The group defends a central warren but can come together with other groups while grazing when they feed in an area known as their home-range which can be up to 50 000 square meters (Lidfors & Edström, 2010). The females construct nests in which the young are born, the nests are visited once daily for a few minutes to feed the young (Lehmann, 1991). After about three weeks the young come out from the nests and they are weaned a week later (Lehmann, 1991). Rabbits are nocturnal animals and wild rabbits feed at dawn and dusk (Gunn & Morton, 1995). Most of the time above ground is spent feeding and they mostly consume grass and herbs (Lidfors & Edström, 2010). They need coarse fibers for their digestion and it is critical to maintain their intestinal flora (Lidfors & Edström, 2010).

Social life and scent marking

In the group there are two separate social hierarchies, one among males and another among females (Vastrade, 1986). The males occupy territories which they defend from one another while the females stay in a specific area but will not defend it against other rabbits (Vastrade, 1986). Lehmann (1991) found that when the rabbits were older than 70 days aggressive and sexual behaviour were common and a linear hierarchy among the males had developed. The alfa male patrols his territory and can be very aggressive towards other males, but he is tolerant with females and young (Lehmann, 1991; Vastrade, 1986). Even though there are frequent aggressive encounters there is always space to retreat and serious injuries are rare (Lehmann, 1991). Rabbits of lower rank can control their interactions by withdrawing to the periphery of the home-range (Held et al., 1995). The alfa male seeks out and interrupts all aggressive or sexual encounters which probably also decrease the risk for injuries. Rabbits also interact more amicable and often lie in close contact with conspecifics while resting (Lehmann, 1991). Nocturnal mammals often use chemical signals to communicate and rabbits has been studied widely in this context (Arteaga et al., 2008). They have a number of scent glands located under the chin and in the anal- and groin regions. They mark their territory with their faeces or by rubbing their chins against objects (Lidfors & Edström, 2010). Chin-marking behaviour has been best studied and it is important for territorial defence and signaling of social dominance (Arteaga et al., 2008). Males and dominant indivividuals scent mark more often than others (Lidfors & Edström, 2010). Males may also spray urine on other rabbits in their social group in order to scent mark them (Lidfors & Edström, 2010).

Rabbits in cages

Rabbits kept in laboratories have traditionally been housed singly in cages. One reason to keep this naturally gregarious species socially isolated has been the problem with aggression among non compatible animals (Morton et al., 1993; Lidfors 1997). The isolation from

conspecifics in this barren environment prevents them from performing several natural behaviours such as digging, allogrooming and some locomotory activities. It also drastically reduces their exposure to variations in odours and diet (Gunn & Morton, 1995). This can lead to the development of abnormal behaviours, eg excessive wall-pawing or bar-gnawing (Gunn & Morton, 1995; Held et al., 1995; Lidfors, 1997; Lidfors & Edström, 2010). Stereotypic behaviour seems to be most frequent at night; this is when rabbits are most active (Gunn & Morton, 1995). Rabbits that are more active tend to become more frustrated and show more abnormal behaviours (Gunn & Morton, 1995). The social isolation has been shown to induce physiological symptoms of stress (Lidfors & Edström, 2010) and individually caged rabbits can also show signs of restlessness (Podberscek et al., 1991) or boredom (Podberscek et al., 1991; Gunn & Morton, 1995; Lidfors & Edström, 2010). Rabbits kept in cages in fact spend a lot of their time inactive and the cage environment is supposed to induce boredom. They have also developed intestinal disorders (Gunn & Morton, 1995) and the limited freedom of movement has been shown to give changes in muscles, bones and joints (Lehmann 1991; Gunn & Morton, 1995; Lidfors & Edström, 2010).

Environmental enrichment

By providing environmental enrichments the amount of abnormal behaviours can be reduced. Straw, hay, chew sticks, cardboard boxes, background noise and taking the rabbits out of the cage for handling or exercise are some things that have been suggested (Lidfors och Edström, 2010). Rabbits spend more time in an area with mirrors and they seem to offer some advantages to their welfare and thus also may be considered an environmental enrichment (Jones & Phillips, 2005; Dalle Zotte et al., 2008). Placing the cages to allow the rabbits to see each other can also be a form of environmental enrichment (Morton et al., 1993). Hay seems to be most important as it is preferred by rabbits and reduces abnormal behaviours in a higher degree than other environmental enrichments; stereotypies may be connected with lack of foraging behaviour (Lidfors, 1997). A raised area should be provided in cages as it reduces abnormal behaviours and nervous responses when being captured (Lidfors & Edström, 2010). It adds structure to the cage and allows the rabbits to move in a way as to maintain normal function and structure of muscles, bones and joints (Stauffacher, 1992). The shelf provides a darker area which can be used when disturbed (Stauffacher, 1992) and function as important hiding from intense light as it can cause retinal damage in albino animals (Lidfors & Edström, 2010).

Group housing

Rabbits should when possible be kept in social groups in pens to meet their need for social behaviour and exercise (Podberscek et al., 1991; Morton et al., 1993; Trocino & Xicatto, 2006; Lidfors & Edström, 2010). Efforts have been made to keep females in groups in pens and breeding females in groups with a male. This has proven successful as rabbits in groups are more active and show no stereotypies (Morton et al., 1993). Rabbits in groups also express a broader behavioural repertoire and when rabbits are kept in groups their quality of life significantly improves even though social stress may lower their welfare (Trocino & Xicatto, 2006; Verga et al., 2007). Held et al. (1995) showed that does have a strong preference for a group pen over a smaller, barren, solitary pen. When group housing it is important to consider the compatability of individual animals, when incompatible rabbits are housed together they will fight and this is especially problematic with males (Morton et al., 1993; Lidfors & Edström, 2010). As males turn aggressive when sexually mature it is difficult to find an alternative to individual caging (Lidfors, 1997). To prevent boredom when group housing is not possible extra care should be taken in order to enrich the animal’s environment (Nevalainen et al., 2007). A rabbit that is caged alone could be given access to a refuge area

with objects to play with (Verga et al., 2007). This is something that has been tried with stud males in some Swedish laboratories as the rabbits are regularly allowed access to an exercise pen with a larger floor surface than their cages (Lidfors & Edström, 2010). All refinements should have verified efficacy on the animals' welfare and be proven safe (Nevalainen et al., 2007). The use of axercise pens have not been scientifically validated and could be both stimulating and stressful (Lidfors & Edström, 2010).

Purpose

The purpose with this study was to evaluate the use of exercise pens as an environmental enrichment for laboratory rabbits by comparing the behaviour and health of animals that had varying access. Questions at issue were:

1. Are there differences between groups that are allowed access to the pens in different degrees?
2. Will the behaviours in the exercise pen change with time?
3. Will behaviours in the cage be affected if the rabbit has access to the exercise pen?
4. Is there a difference in the behaviours expressed in the exercise pen compared to the cage?
5. Does access to exercise pens affect the weight of the rabbits?
6. Does access to exercise pens affect corticosterone levels?
7. Does access to exercise pens affect general health parameters?

Materials and methods

The study was carried out at Astra Zeneca R&D, Safety Assessment, Södertälje.

Animals, housing and management

Twenty one male New Zealand White rabbits (Charles River Deutschland GmBH, Germany, substrain Crl:KBL(NZW)BR) of the age 4 months – 3 years were housed individually. They had been kept there for at least 4 weeks before start of the study and were used as stud males, used for mating in reproductive toxicology studies. The rabbits weighed 3.2 – 5.4 kg at the start of the study.

Each animal was uniquely identified by an animal number, using an ear tattoo. They were also uniquely identified within the study by an animal reference number. The correlation between the individual animal number and the animal reference number was documented in the raw data.

The animals were singly housed in cages with stainless steel walls with perforated polypropylene floors over paper-lined trays placed in rolling racks (Scanbur EC2, Danmark). Each rabbit had a double cage so that the living area was 2 x 5200 cm^2. Cages contained removable shelves. The paper lining was changed twice a week or more frequently if needed. The polypropylene floors and removable shelves were changed for cleaning when considered necessary.

The animals were given fresh water and pelleted food once daily (K1 Special, Lantmännen, Lidköping, Sweden). Once a week the water bottles were dished. The diet was analysed for nutrients, and both diet and water were analysed for chemical and microbial contaminants. Target values for temperature and relative humidity were 14 to 20°C and 40 to 70%, respectively. The animal room was illuminated by artificial light from fluorescent tubes on an approximately 16 hour/8 hour light/dark cycle. There were no windows. Each animal was given autoclaved hay daily and provided with aspen wood chew blocks. Environmental records and Certificates of Analysis for diet and water were stored centrally in the archives of Safety Assessment Sweden, Södertälje.

Most of the rabbits had been given exercise earlier in forms of running freely in an enclosure on the floor in the room. This was not done regularly, and they did not have a plastic, anti slip, carpet or enrichment objects.

Study design

The rabbits were allocated to treatment groups so that each group contained rabbits of different ages. Each animal cage was provided with a colour coded label bearing all the information necessary to identify the animal in the cage. Animals were placed randomized in the room so that all animals in one group would not live in the same part of the room.
The treatment groups were:

- Controls: No exercise. Kept in their cages the whole study except when being weighed and taken blood sample on.
- EX1/W: Placed in one of the exercise pens during 1 hr on 1 day/week (Tuesdays).
- EX3/W: Placed in one of the exercise pens during 1 hr on 3 days/week (Monday, Wednesday and Friday).

Exercise sessions were carried out during week 1-8 between 08.00 and 16.00 hours. Number of animals and the frequency of which they were given access to exercise pens are included in Table 1.The first day with exercise sessions was designated as Day 1 of the study and the first seven days were Week 1. The day before the exercise sessions started was Day -1.

Table 1. Treatments, number of animals, animal numbers and frequency of exercise

Treatment	*Animals*	*Animal reference numbers*	*Frequency of exercise*
Controls	7 Males	1 to 7	None, control
EX1/W	7 Males	8 to 14	1 hr/week
EX3/W	7 Males	15 to 21	3*1 hr/week

Three exercise pens were made from plastic coated steel bar frames which enabled an oversight of the whole pen. The pens were situated in the middle of the room where the animals lived and with a distance of about 30 cm from one another in order to disable any near contact between the rabbits. They stood in a row so that the long side of one pen was opposite of the long side of the next. The pens were 3.65 m long, 0.9 m wide and 0.76 m high. Each pen stood on a rubber carpet to make the floor less slippery. On one short side there was a combined shelf/hide identical with the ones used in the cages. On the other side was a plastic box (BK Rat Cage (large rat cage), 1300 cm^2 and 21 cm high) filled with wood shavings and hay, approximately 750 g of wood shavings and 150 g hay. The pen also contained a watter bottle and a wood chew block like the ones in their cages. They also had a plastic toy, a ball with 3 holes (Crawlball, transparent polycarbonate, PLEXX BV, Netherlands).

Figure 1. The three exercise pens.

The groups that had access to the exercise pens were divided into subgroups of 2-3 animals that was exercised at the same time and one specific animal was always in the same pen. The subgroups were exercised in different order on different days after a schedule so that one specific rabbit had access to the pen on a different time each session.

Clinical observations and measurements

All animals were thoroughly examined during week -1. Animals were checked and clinical observations were recorded at least once daily during the study period. Body weights of all animals were recorded midday every Tuesday during the study period (Satorius, Model 12, GMBH Göttingen, Germany). Food and water consumption was monitored during the study period. If animals left any food in the food trough or drank less than 100 ml in one day it was recorded.

Behavioural Observations

Behaviour in the exercise pens began to be recorded when all the rabbits of one subgroup had entered their pens (for EX3/W during the first session of the week) and continued during the whole stay. While recordings were made none other than the observer was in the room. The observer sat on a chair about one meter from the short side of the pens on one side. The same observer recorded behaviour for all animals during the whole study. Once a minute the occurrence of one of 9 defined behaviours was recorded instantaneously (Table 2). A stopwatch was set to ring every minute, and each time it rang it was recorded what all the rabbits were doing in their pens watching the pens in the same order every time. It was also recorded where the rabbit was, i.e. on the shelf, under the shelf, in the box with wood shavings or at any other place in the pen. In addition the frequency of 14 defined behaviours

(Table 3) was recorded during one minute in the beginning of the session, one minute in the middle and one minute in the end.

Table 2. Description of behaviours recorded once a minute in pens and every second minute in cages

Behaviour	*Description*
Lying	On the side or on the chest with the legs under the body, might be alert or dozed.
Sitting	More upright than the previous, the forelimbs not folded under the body so that the thorax is clear from the floor, all paws are in contact with the floor.
Eating	Chewing hay, pellets or faecal pellets
Drinking	Lapping water from the nipple of the water bottle.
Hiding	Lying flat and stiff on the ground, hindlegs pressed under the body and ears pressed against the back.
Grooming	Licking the body, pulling of the forepaws over the head or scratching its body with a hind foot.
Gnawing	Chewing, biting, pulling and nibbling of the wooden block, the plastic toy, the box with wood shavings, the shelf/hide or other parts of the cage/pen, except for hay or feed pellets.
Moving	Movement around the pen/cage, includes crawling, hopping, jumping, frisky hop.
Other behaviours	Behaviours not mentioned above, eg. rearing, urinating, marking territory, digging.

Table 3. Description of behaviours recorded during three minutes per exercise session

Behaviour	*Description*
Rearing	Standing up, front paws not in contact with the ground.
Leaping	Very rapid running around, frisky hop or leaping, sometimes shaking the body or head or kicking with the hind feet.
Digging	Scratching with the forepaws in the box or on another surface.
Playing toy	Tossing the toy around, punching it with the front legs or pushing it ahead of itself with their heads.
Gnawing block	Chewing, biting, pulling and nibbling of the wooden block.
Gnawing cage/pen	Chewing, biting, pulling and nibbling of the plastic toy, the box with wood shavings, the shelf/hide or other parts of the cage/pen, except for hay or feed pellets.
Stretching	Extending the forepaws while tilting the head backwards or extending upwards on its limbs while arching the back, sometimes while yawning.
Grooming	Licking the body, pulling of the forepaws over the head or scratching its body with a hind foot.
Eating	Chewing hay, pellets or faecal pellets.
Drinking	Lapping water from the nipple of the water bottle.
Marking territory	Chin-marking (rubbing the chin over a surface) or enurinating (twisting of the hindquarters while emitting a jet of urine).
Urinating	Emitting urine (not enurinating).
Parading	Follows neighbour along the fence, tail held high and stiff movements, sometimes growling/humming.
Investigatory behaviour	Sniffing its environment, either different parts of the cage/pen or the air with its nose pressed out between the bars, nose twitching.

Behaviours in the cages were observed during week 1, 3, 4, 5 and 8 during Thursdays when there were no exercise sessions. All animals were observed during 2 hours sometime between 09.00 and 16.00. Every second minute the same behaviours as in the exercise pens (Table 2) were recorded instantaneously in each rabbit in order from the first cage to the last cage. The behaviours that were recorded can be found in Table 2. It was also recorded where the rabbit was, i.e. on the shelf, under the shelf or on the cage floor. During one day the rabbits were observed in the cages while other rabbits were being exercised. This was done to check whether their behaviours were affected. This was not planned from the beginning but the question of how much they were affected was raised during the course of the study.

Clinical pathology

Blood was collected from non-fasted animals. All animals were bled and samples were collected from the central ear artery on day -5, 2 or 3, 23 or 24 and 51 or 52. The blood samples were collected before and after exercise as the rabbits were taken to or from the exercise pens at 8.00-16.00. Two subgroups (4-5 rabbits) were exercised in the morning and the third subgroup in the afternoon (2-3 rabbits), in connection with samples being taken from the exercised rabbits samples were also taken from a corresponding number of controls.Samples from the controls were thus taken at a corresponding time, between 8.00-10.00 and 10.30-14.15.

Approximately 0.6 mL were collected in lithium heparin for analysis of plasma chemistry parameters and 0.5 mL in EDTA for analysis of haematology parameters on day -5, 51 and 52. Samples were analysed for the parameters included in Table 4.

Table 4. Blood parameters analysed from control rabbits and EX1/W and EX3/W before exercise (day -5)and after 8 weeks of exercise (day 51-52)

Haematology	*Plasma chemistry*
Erythrocytes	Alanine aminotransferase
Haemoglobin	Alkaline phosphatase
Haematocrit	Aspartate aminotransferase
Mean red cell haemoglobin	Bilirubin (total)
Mean red cell haemoglobin concentration	Calcium
Mean red cell volume	Cholesterol
Red cell distribution width	Creatinine
Reticulocytes	Globulin
Platelets	Glucose
Leucocytes	Glutamate dehydrogenase
Neutrophils	Potassium
Lymphocytes	Sodium
Monocytes	Total protein
Basophils	Triglycerides
Eosinophils	Urea
Large unstained cells	

In addition approximately 0.6 mL was collected in tubes without anticoagulant for analysis of corticosterone. These levels were analysed using Corticosterone ELISA Kit catno K3014-1 (B-Bridge International Inc, US). The assay uses an antibody sandwich to link corticosterone to the microtiter plate. Levels of coricosterone in samples are measured by competition with

known amounts of a detectable coricosterone-peroxidase conjugate. The procedure is straight forward and involves only two incubations: a one hour incubation to capture the corticosterone complexes followed by a second 30 minutes incubation to develop the detection reagent. The microtiter plate has been coated with antibodies that recognize sheep antibodies. Sheep anti-corticosterone antibodies are then used to capture corticosterone and bind it to the plate. Corticosterone levels in the samples are quantified using a corticosterone-peroxidase conjugate. Corticosterone in the samples competes with the corticosterone-peroxidase conjugate. After addition of substrate, the assay signal decreases with increasing amounts of corticosteron. A corticosterone standard is provided to generate a standard curve for the assay and all samples should be read of the standard curve. The standard concentrations were 78.125; 156.25; 312.5; 625; 1250; 2500 and 5000 pg/mL, prepared with a serial dilution from a stock solution.

All serum samples were diluted in two steps the first step 1:2 with dissociation reagent and the second 1:20 with assay buffer. 50 µL standard samples and control were pipetted to the microtiter plate. 75 µL assay buffer were pipetted to two wells for the non specific binding. 25 µL of corticosterone –peroxidase conjugate were added to the plate except the non specific binding. The plate was incubated one hour on a plate shaker at room temperature A washing step including 4 cycles were performed. 100 µL TMB were added to all wells and another 30 minutes incubation without shaking was performed. The reaction was stopped by adding 50 µL stop solution to all wells. Absorbancances were read at 450nm with a reference wavelength of 570 nm. Equipment used were: Heidolph Titramax microtiterplate shaker, Columbus Pro microtiterplate washer from Tecan and Sunrise absorbance reader with Magellan software from Tecan.

Statistical analysis

Behavioural data

Statistical objectives were to summarise the evidence of treatment related changes in the proportion of occasions that the rabbits where doing one of the following activities: Moving, sitting, lying, grooming and eating. Because the analysis was done on proportions rather than on the original count data, it is reasonable to assume the normal distribution of the proportions. A linear statistical model was fitted to the data from the two dataset separately, using *Treatment group*, *Age, Week of assessment,* and the interaction between the week of assessment and treatment group as the explanatory variables. Age was entered as a binary variable with ages under 1 year and those equal to or greater than 1 year in two different categories. The interaction term between the week of assessment and treatment group was used to test for differences between the treatment groups over time. In order to test whether the relationship between the treatment groups with time (week of exercise) was different between the two datasets, the additional 3-way interaction between *Treatment group*, *Week of assessment*, and *Dataset* (Burobs, Interval) was tested in a linear model constructed using the combined data for the two assessments. For the dataset of frequency of some behaviours, the total numbers of occasions for the following activities were analysed: marking territory, leaping, rearing, social behaviour and digging. The statistical models used were similar to that described above, and differences for the frequency of each activity between the treatment groups, per assessment week, were compared.

Clinical pathology data

For the corticosterone dataset, the differences in levels within each treatment group for assessment weeks 1, 4 and 8 were compared to the level for week -1 using a series of pair-

wise t-tests. The method outlined here is however an improvement on simple pair-wise testing, as it takes advantage of the repeated measures on each animal to derive a more robust estimate of the standard error of the mean changes. The dataset for clinical pathology was analysed in a similar manner and the differences in levels within each treatment group for week 8 were compared to the level for week -1 using a series of pair-wise t-tests. The weight dataset was also analysed in a similar manner but the difference was that all groups were compared for all points in time. Results were adjusted for age was included as a covariate in the analysis.

Results

Behaviour in cages

The most common behaviours performed in the cages were lying, and then came eating, sitting, grooming, moving and drinking as relatively common behaviours (Table 5). There were large individual differences in how often some behaviours occurred, e.g. lying (29-82%), and some behaviours such as moving and gnawing were almost never performed by some rabbits (Table 5).

Table 5. Mean percentages (SE) of different behaviours shown in cages by male NZW rabbits when exercised once per week (EX1/W), three times per week (EX3/W) or not at all (controls)(n=7 rabbits/treatment). The lowest (Min) and the highest (Max) percentage of recordings per individual rabbit over all treatments

Behaviour	Controls	EX1/W	EX3/W	Min	Max
Lying	48.8 (6.8)	57.3 (4.6)	53.8 (3.4)	29.4	82.3
Eating	20.1 (4.8)	14.7 (2.4)	13.1 (1.2)	5.5	42.6
Sitting	12.5 (1.9)	9.0 (1.5)	13.2 (1.8)	4.5	21.6
Grooming	9.3 (1.1)	10.5 (1.2)	8.9 (1.2)	4.8	13.9
Moving	5.1 (1.2)	3.3 (0.9)	7.1 (1.8)	0.3	15.8
Drinking	3.5 (0.6)	3.7 (1.2)	3.0 (0.4)	0.6	10.3
Other	0.6 (0.1)	1.1 (0.4)	0.8 (0.3)	0	3.2
Gnawing	0.1 (0.1)	0.3 (0.3)	0.1 (0.1)	0	1.9

Only minor differences between the treatment groups were noted in the occurrence of different behaviours over the entire study period and none were significantly different (Figure 2).However, when comparing treatment groups within each week it was found that during week 5 rabbits in the control group had a significantly higher number of recordings for eating than both rabbits exercised once per week ($p<0.05$) and three times per week ($p<0.05$). They also ate significantly more than those exercised once per week during week 8 ($p<0.05$) and there was a tendency that they ate more than rabbits exercised three times per week during the same week ($p=0.0559$). During week 8 rabbits exercised three times per week had a higher percentage of moving than both controls ($p<0.05$) and rabbits exercised once per week ($p<0.005$). During this week rabbits exercised once per week had a higher percentage of lying than rabbits in the control group ($p<0.05$) while the controls had a higher percentage of sitting than those exercised once per week ($p<0.05$). Rabbits exercised three times per week had a higher number of recordings for sitting than rabbits exercised once per week during week 1 ($p<0.05$). No differences between the groups could be detected for grooming.

No hiding behaviour was recorded in the cages.

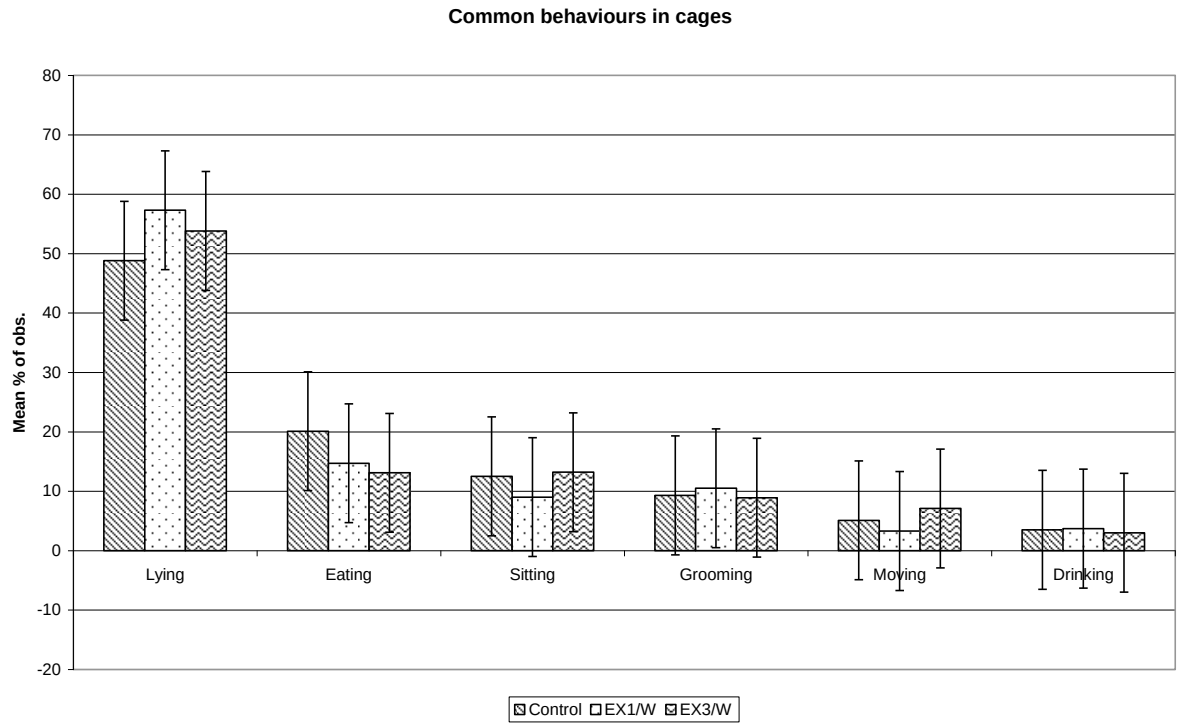

Figure 2. Mean percentages (± SE) of different behaviours shown in cages by male NZW rabbits when exercised once per week (EX1/W), three times per week (EX3/W) or not being exercised (controls (n= 7 rabbits/treatment).

Young rabbits had a significantly higher number of recordings for eating ($p<0.05$) in the cages. Older rabbits instead had a significantly higher percentage of lying ($p<0.05$).No age differences could be detected for sitting, grooming and moving.

Placement in cages

The most common placement of the rabbits was on the cage floor followed by on the shelf and less commonly under the shelf. There were large individual differences in where the rabbits were in the cage, e.g. on the shelf (0-51%).

Rabbits in the control group appeared to have a higher number of recordings on the shelf whereas rabbits exercised once per week had a higher percentage under the shelf. However, none of these differences were significant, probably due to the large individual differences.

Table 6. Mean percentages (SE) of different placements in cages of male NZW rabbits when exercised once per week (EX1/W), three times per week (EX3/W) or not at all (controls)(n=7 rabbits/treatment). The lowest (Min) and the highest (Max) percentage of recordings per individual rabbit over all treatments

Placement	Controls	EX1/W	EX3/W	Min	Max
On Shelf	18.3 (7.2)	7.6 (2.8)	13.3 (4.5)	0	50.6
Under Shelf	6.5 (4.6)	9.9 (5.2)	7.2 (4.5)	0	39.4
Other place	75.3 (6.3)	82.5 (5.4)	79.5 (4.4)	48.7	100

Behaviour in pens

General behaviour

The most common behaviour performed in the pens were moving, and then came sitting, lying, grooming and eating as relatively common behaviours (Table 7). There were large individual differences in how often some behaviours occurred, e.g. moving (23-59%), and some behaviours such as lying and hiding were never shown by some rabbits (Table 7).

Table 7. Mean percentages (SE) of different behaviours shown by male NZW rabbits when exercised either once per week (EX1/W) or three times per week (EX3/W) for one hour (n=7 rabbits/treatment). The lowest (Min) and the highest (Max) percentage of recordings per individual rabbit over all treatments

Behaviour	EX1/W	EX3/W	Min	Max
Moving	50.4 (4.7)	49.7 (2.1)	23.1	59.1
Sitting	16.1 (2.9)	15.6 (1.7)	6.5	31.3
Lying	5.2 (2.8)	13.3 (3.2)	0	23.8
Grooming	8.1 (1.4)	5.1 (1.3)	2.3	12.7
Eating	7.9 (2.3)	5.4 (2.1)	1.0	17.7
Hiding	1.1 (0.9)	0.6 (0.3)	0	6.7
Gnawing	0.5 (0.2)	0.7 (0.2)	0	1.5
Drinking	0.1 (0.1)	0.1 (0.1)	0	0.6
Other	10.5 (2.0)	9.5 (1.4)	4.6	16.9

The only significant difference found in the behaviour of the rabbits over the entire study period were that the rabbits exercised three times per week had a higher percentage of lying than those exercised once per week ($p<0.05$, Figure 3). When comparing within each week the rabbits exercised three times per week had a higher percentage of lying during week 2 and 4 than the rabbits exercised once per week ($p<0.05$). When comparing mean percentages it looks as if the rabbits exercised once per week were eating and grooming more, but there were no significant differences over the entire study period ($p= 0.405$, $p=0.055$). However, rabbits exercised once per week had a significantly higher percentage of eating during week 4 ($p<0.05$) and for grooming during week 2 and 4 ($p<0.05$, $p<0.05$). Rabbits exercised once per week moved significantly more than rabbits exercised three times per week during the first week ($p<0.05$) but there was no difference between the groups in the following weeks, rabbits exercised three times per week instead sat more during the first week ($p<0.01$).

Hiding occupied around 0.5-1% of the time, and almost all of this behaviour occurred during the first exercise session. Three rabbits in EX1/W drank water but only one did it twice.

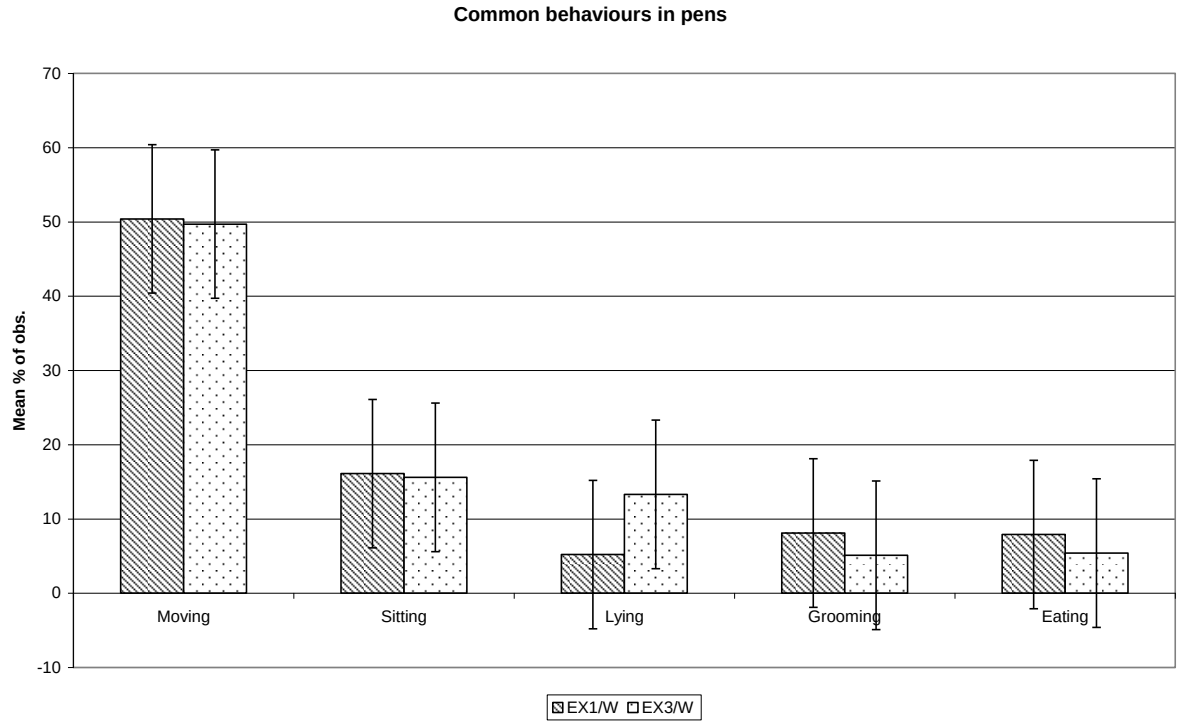

Figure 3. Mean percentages (± SE) of different behaviours shown by male NZW rabbits when exercised once per week (EX1/W) or three times per week (EX3/W) for one hour

Older rabbits had a significantly higher percentage of grooming ($p<0.05$), and tended to have a higher percentage of lying ($p=0.65$). No age differences could be detected for moving, sitting and eating.

Frequency of some behaviours

When the frequency of performing a specific behaviour was recorded it was found that investigatory behaviour was clearly most common (Table 8). Marking territory came in second, and other less common behaviours were leaping, rearing, parading, digging and gnawing (Table 8). There were large individual differences but investigatory behaviour, marking territory and rearing was performed by all animals at least once (Table 8).

Table 8. Mean number of times (SE) different behaviours were shown by male NZW rabbits when exercised either once per week (EX1/W) or three times per week (EX3/W) for 3 minutes during 8 exercise sessions (24 minutes) (n=7 rabbits/treatment). The lowest (Min) and the highest (Max) percentage of recordings per individual rabbit over all treatments

Behaviour	EX1/W	EX3/W	Min	Max
Investigatory behaviour	47.9 (6.6)	61.3 (4.2)	11	74
Marking territory	18.7 (4.9)	31.9 (4.4)	1	52
Leaping	13.7 (3.3)	13.1 (2.5)	0	26
Rearing	16.3 (2.7)	10.0 (1.5)	5	23
Parading	3.4 (1.7)	7.7 (4.2)	0	31
Digging	3.6 (1.6)	6.4 (1.2)	0	12
Gnawing	2.7 (1.3)	2.6 (1.5)	0	11

No significant differences could be found in the number of recordings of the seven behaviours of the rabbits over the entire study period between the rabbits exercised once or three times per week (Figure 4). However, rabbits exercised three times per week had a significantly higher number of recordings for marking territory during week 2 and 7 ($p<0.05$, $p=0.01$). Rabbits exercised once per week on the other hand had higher number of recordings for rearing during week 6 ($p<0.05$) and leaping during week 7 ($p<0.05$). No differences between the treatment groups could be detected for parading and digging.

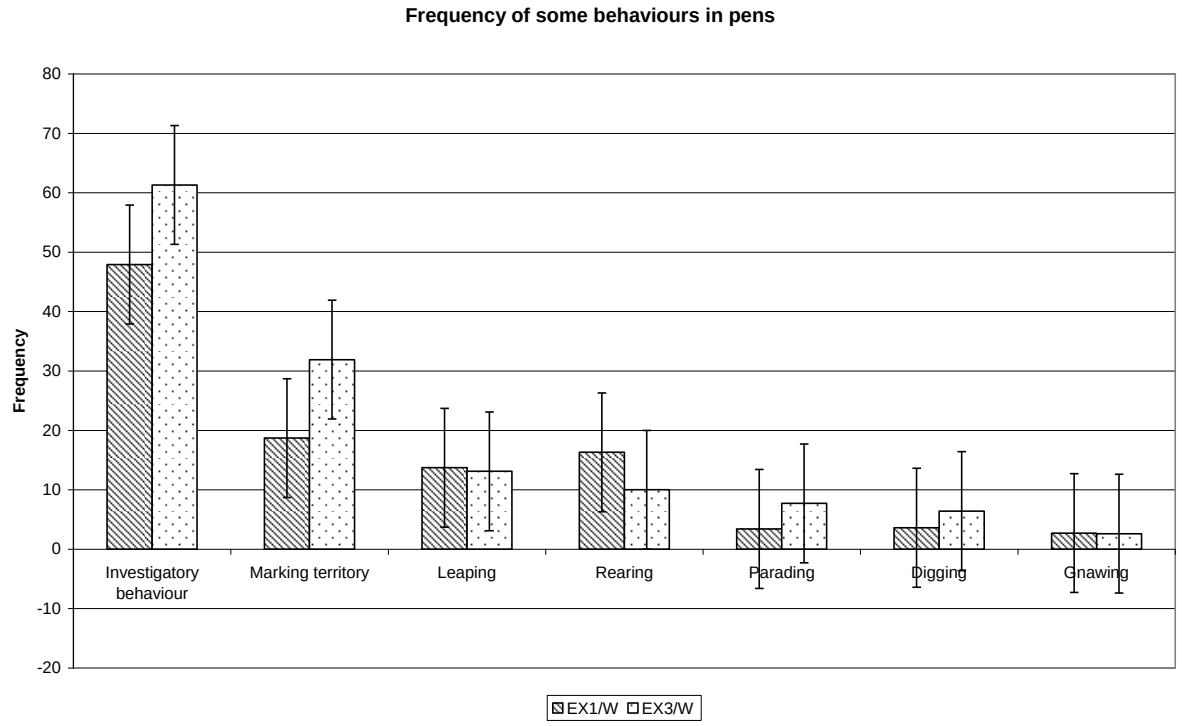

Figure 4. Mean frequency (± SE) of some behaviours shown by male NZW rabbits when exercised once per week (EX1/W) or three times per week (EX3/W) for one hour

During the three minutes observations per exercise hour rabbits were never recorded to interact with the ball. However, six of the rabbits interacted with the ball, but these interactions occurred between recordings and are not included in any results. Only three of these six rabbits interacted with the ball more than once during the study period and the plastic ball was maximally interacted with 3 times by one rabbit. Four of the six rabbits who interacted with the ball and all who engaged with it more than once were rabbits being exercised once per week.

The chewing stick was never recorded to be gnawed on. However, between recordings one rabbit was observed to gnaw on it twice. All recorded gnawing (Table 8) is performed on other parts of the pen and not the chewing stick. The rabbits neither stretched nor yawned during the observations and only one urinated in a non-marking way.

Young rabbits had a significantly higher number of recordings for leaping ($p<0.05$) and rearing ($p<0.005$) in their pens. No age differences could be detected for parading, marking territory and digging.

Placement in pens

The most common placement of the rabbits were in the open areas of the exercise pen, i.e. neither by the shelf nor the box (Table 9). After that they were in the box, on the shelf and the least under the shelf (Table 9). Also here there were large individual differences, e.g. in the box (0-88%), but all places in the pen except for on the shelf were visited by all rabbits at least once.

Rabbits exercised three times per week appeared to have a higher number of recordings on the shelf whereas rabbits exercised once per week had a slightly higher percentage in the box. However, none of these differences were significant, probably due to the large individual differences.

Table 9. Mean percentages (SE) of different placements of male NZW rabbits when exercised either once per week (EX1/W) or three times per week (EX3/W) for one hour (n=7 rabbits/treatment). The lowest (Min) and the highest (Max) percentage of recordings per individual rabbit over all treatments

Placement	EX1/W	EX3/W	Min	Max
Open area	60.7 (9.3)	68.5 (5.9)	10.4	90.0
In Box	27.5 (11.5)	14.8 (4.2)	0.4	88.3
On Shelf	5.6 (3.0)	10.5 (5.0)	0	36.0
Under Shelf	6.2 (2.6)	6.2 (1.7)	0.4	16.9

Comparison of behaviours between cages and pens

Moving was the behaviour most commonly performed in the pens with around half of the observations, whereas in the cages it took up less than a fifth of that amount (Figure 5). There was a significant difference in the amount of moving between the three groups over time caused by the exercise ($p<0.05$). Lying on the other hand was the behaviour most commonly performed in the cages with around half of the observations, but in the pens it was only performed during 5-13% of the observations. A difference in percentage of obs. lying between the three groups over time caused by the exercise could not be detected. Sitting was performed during approximately 16% of the observations in the pens and during 9-13% in the cages. Grooming was performed during approxiamtely 5-8% of the observations in the pens and during 9-11% in the cages There was a significant difference in both the amount of sitting ($p<0.05$) and grooming ($p<0.05$) between the three groups over time caused by the exercise. Eating was performed during approximately 5-8% of the observations in the pens and during 13-20% in the cages. A difference in percentage of obs. eating between the three groups over time caused by the exercise could not be detected.

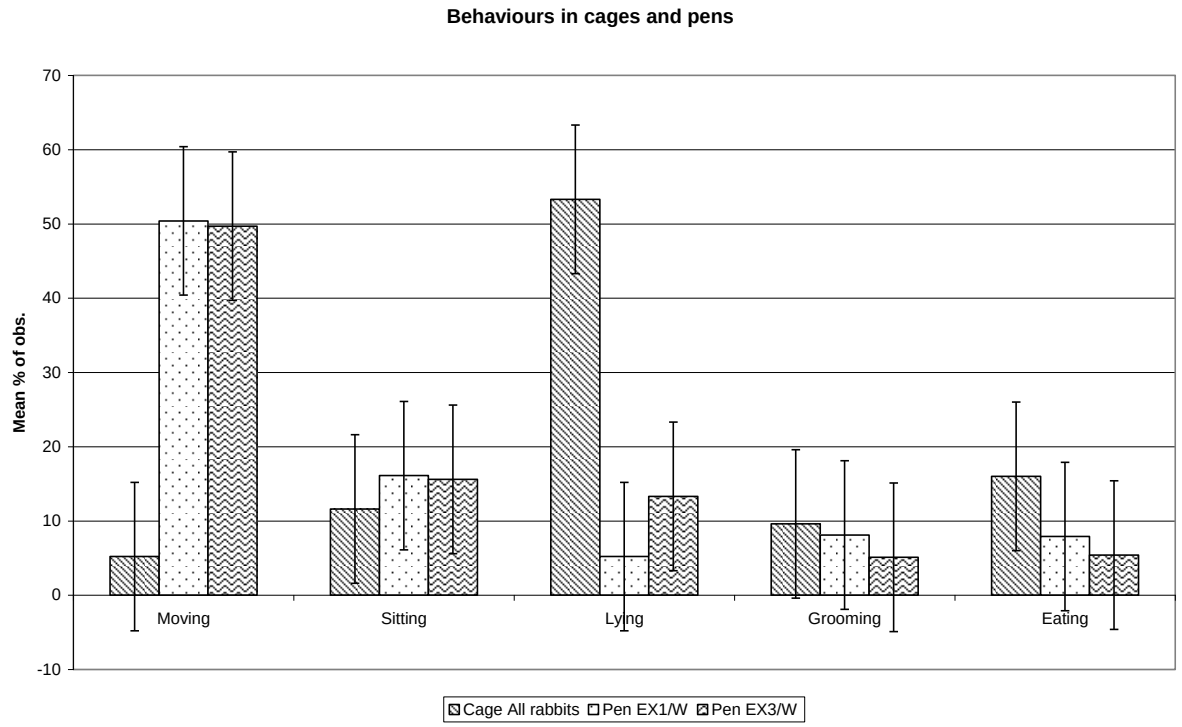

Figure 5. Mean percentages (± SE) of different behaviours shown in cages and pens by male NZW rabbits. Comparison between all rabbits in cages and rabbits that have been exercised once per week (EX1/W) and three times per week (EX3/W) for one hour (n=7 rabbits/treatment).

Behaviour in cages during exercise sessions

The behaviour performed in cages seemed to be affected by other rabbits being exercised. An increase could be seen in behaviours such as moving, other behaviours (eg. digging, marking territory and rearing), gnawing, eating and grooming (Figure 6). Lying, sitting and drinking decreased during times when other rabbits were being exercised.

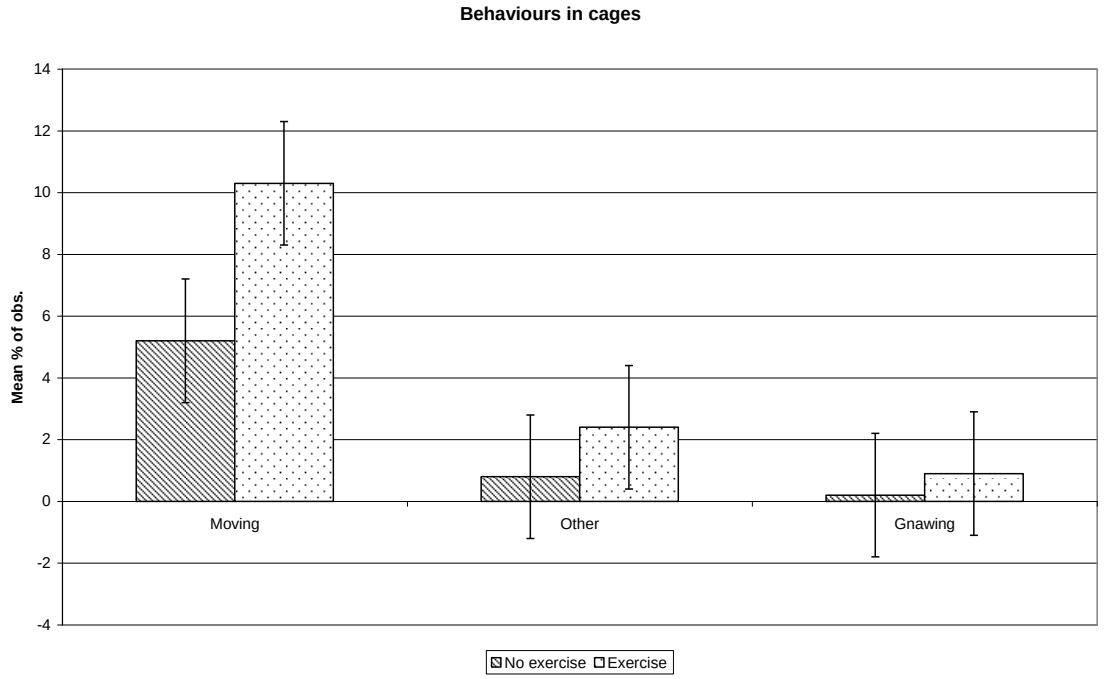

Figure 6. Mean percentages (± SE) of different behaviours shown in cages by male NZW rabbits during exercise sessions compared to during no exercise sessions.

Even though rabbits in all groups seemed to be affected by other rabbits being exercised they may not be affected to the same degree. Rabbits exercised once or three times per week had a higher increase of moving than rabbits in the control group during times when other rabbits were being exercised (Figure 7).

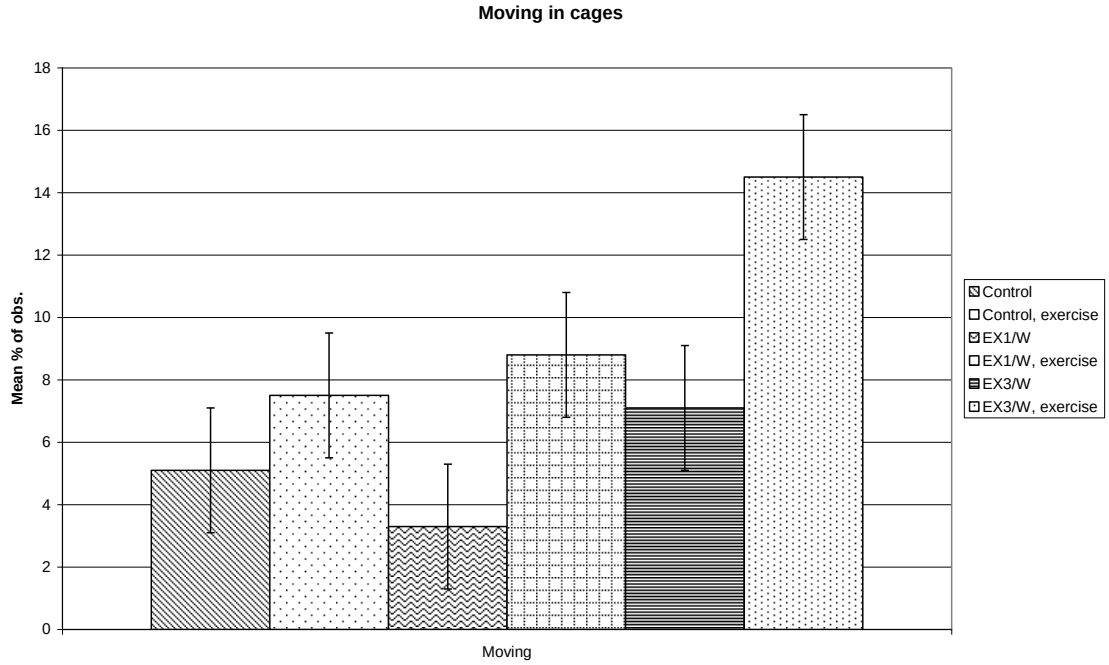

Figure 7. Mean percentages (± SE) of moving in cages by male NZW rabbits during and outside exercise sessions, comparison between rabbits that have been exercised once per week (EX1/W), three times per week (EX3/W) and controls which have not been exercised.

Clinical recordings

Weight

Rabbits under one year gained weight during the study period while older rabbits lost weight (Figure 7). There was no major difference in the weight gain between the rabbits in the control group (7.8%), those exercised once per week (6.4%) and those exercised three times per week (7.9%). Older rabbits in the control group lost weight (1.5%), but not to the same amount as those exercised. There was no major difference between the rabbits exercised once or three times per week (5.0%, 4.8% weight loss). However, none of these differences were significant, probably due to the small groups that had to be made because of the age difference in each exercise group.

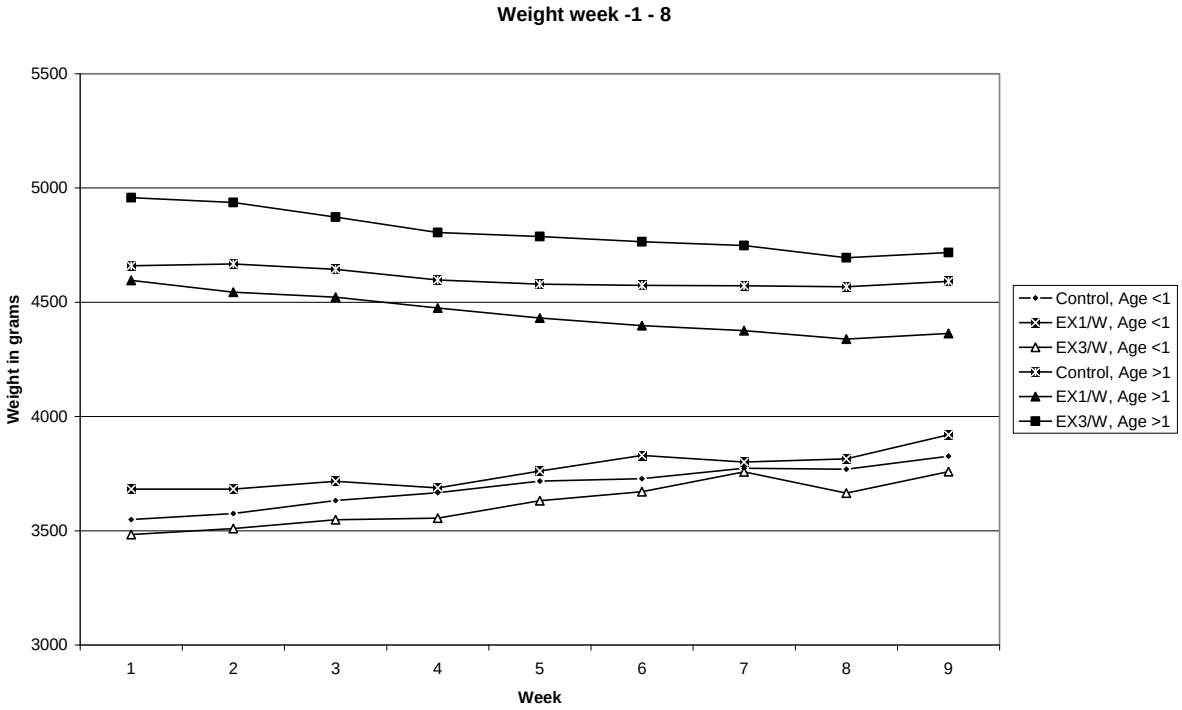

Figure 8. Mean weight in grams for rabbits in the control group, exercised once per week (EX1/W) or three times per week (EX3/W), divided into groups over and under 1 year of age during week -1 to 8 (n=3 rabbits Control, Age<1; 3 rabbits EX1/W, Age<1; 2 rabbits EX3/W, Age<1; 4 rabbits Control, Age>1; 4 rabbits EX1/W, Age>1; 5 rabbits EX3/W, Age>1).

Corticosterone

Corticosterone levels in plasma were elevated ($p<0.005$) after exercise during the first week (week 1) compared to the week before exercise started (week -1) for rabbits exercised once per week (Table 10). No significant difference could be detected in the samples taken before exercise or for the other groups. During week 4 and 8 with exercise no significant differences in corticosterone were found compared to week -1.

Table 10. Mean values of corticosterone in ng/ml (SD) in blood samples from male NZW rabbits when exercised either once per week (EX1/W) or three times per week (EX3/W) or not at all (controls). Samples taken before exercise in the morning and after exercise in the afternoon

		EX1/W	EX3/W	Controls
Morning (before exercise)	Week -1	13.3 (6.3)	8.2 (2.0)	12.0 (1.5)
	Week 1	11.1 (3.3)	12.5 (7.2)	11.3 (3.3)
	Week 4	18.8 (7.1)	11.3 (2.9)	13.8 (3.4)
	Week 8	19.1 (12.0)	11.7 (2.0)	16.3 (8.0)
Afternoon (after exercise)	Week -1	17.2 (14.6)	13.6 (3.3)	15.4 (5.5)
	Week 1	58.8 (35.5)	32.4 (31.5)	30.2 (42.9)
	Week 4	24.3 (8.6)	18.9 (7.7)	15.4 (2.6)
	Week 8	15.4 (2.2)	19.7 (8.8)	24.9 (25.2)

Haematology and plasma chemistry

Only minor changes in mean values of general blood parameters could be detected between the different groups and none could be interpreted as an effect from the exercise sessions (Table 11 and 12).

Table 11. Mean values of haematology parameters (SD) in blood samples from male NZW rabbits when exercised either once per week (EX1/W) or three times per week (EX3/W) or not at all (controls). Samples taken before exercise (day -5)and after 8 weeks of exercise (day 51-52)

Parameter	EX1/W week -1	EX1/W week 8	EX3/W week -1	EX3/W week 8	Controls week -1	Controls week 8
BASO	0.34 (0.09)	0.34 (0.08)	0.36 (0.11)	0.32 (0.11)	0.36 (0.09)	0.36 (0.07)
EOS	0.13 (0.04)	0.14 (0.03)	0.16 (0.03)	0.13 (0.02)	0.11 (0.04)	0.11 (0.04)
HCT	40 (1)	41 (2)	39 (2)	41 (2)	38 (1)	39 (1)
HGB	140 (5)	139 (7)	138 (6)	136 (9)	133 (2)	133 (5)
LUC	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
LYM	5.08 (0.91)	4.67 (1.25)	5.23 (1.37)	4.38 (1.54)	6.04 (1.79)	5.28 (2.09)
MCH	22.7 (0.8)	22.6 (0.8)	22.9 (0.4)	22.7 (0.5)	22.5 (1.2)	22.5 (1.3)
MCHC	349 (4)	339 (6)	351 (4)	337 (3)	347 (3)	340 (7)
MCV	65.1 (2.2)	66.8 (2.0)	65.2 (1.8)	67.3 (1.4)	64.9 (3.3)	66.1 (3.7)
MONO	0.03 (0.02)	0.19 (0.03)	0.05 (0.01)	0.33 (0.13)	0.05 (0.03)	0.22 (0.08)
NEUT	1.05 (0.34)	1.31 (0.39)	1.33 (0.39)	1.50 (0.57)	1.10 (0.37)	1.21 (0.29)
PLT	415 (98)	341 (68)	328 (41)	308 (34)	447 (179)	363 (127)
RBC	6.15 (0.28)	6.15 (0.25)	6.05 (0.23)	6.01 (0.39)	5.90 (0.32)	5.92 (0.41)
RDW	12.9 (0.5)	13.1 (0.5)	12.6 (0.3)	12.9 (0.4)	13.0 (0.5)	13.0 (0.6)
RET	134 (18)	141 (21)	124 (16)	134 (16)	150 (30)	134 (20)
WBC	6.65 (1.18)	6.65 (1.17)	7.13 (1.21)	6.67 (1.59)	7.68 (2.05)	7.18 (2.33)

Parameter Explanation
BASO – Basophils 10^9/L
EOS – Eosinophils 10^9/L
HCT - Haematocrit percent
HGB – Haemoglobin g/L
LUC – Large unstained cells 10^9/L
LYM – Lymphocytes 10^9/L
MCH – Mean Cell Haemoglobin pg
MCHC – Mean Cell Haemoglobin Conc g/L
MCV – Mean Cell Volume fL
MONO – Monocytes 10^9/L

NEUT – Neutrophils 10^9/L
PLT – Platelets 10^9/L
RBC – Red Blood Cell Count 10^12/L
RDW – Red Cell Distribution Widht %
RET – Reticulocytes 10^9/L
WBC – White Blood Cell Count 10^9/L

Table 12. Mean values of clinical chemistry parameters (SD) in blood samples from male NZW rabbits when exercised either once per week (EX1/W) or three times per week (EX3/W) or not at all (controls). Samples taken before exercise (day -5)and after 8 weeks of exercise (day 51-52)

Parameter	EX1/W week -1	EX1/W week 8	EX3/W week -1	EX3/W week 8	Controls week -1	Controls week 8
Parameter	57 (35)	42 (18)	51 (40)	31 (13)	68 (43)	42 (17)
ALP	57 (35)	42 (18)	51 (40)	31 (13)	68 (43)	42 (17)
ALT	35 (12)	64 (28)	36 (17)	51 (27)	37 (9)	57 (25)
AST	16 (5)	39 (27)	16 (4)	22 (7)	19 (12)	23 (10)
CA	3.67 (0.12)	3.55 (0.09)	3.60 (0.10)	3.48 (0.11)	3.67 (0.18)	3.55 (0.17)
CHOL	0.6 (0.1)	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	0.6 (0.1)	0.6 (0.2)
CREA	78 (14)	75 (7)	82 (12)	73 (7)	78 (4)	71 (7)
GLDH	8 (2)	16 (8)	8 (3)	13 (3)	10 (5)	9 (3)
GLU	7.9 (0.6)	7.7 (0.5)	7.6 (0.3)	7.6 (0.4)	7.5 (0.3)	7.5 (0.4)
K	4.1 (0.2)	4.0 (0.3)	4.2 (0.4)	4.2 (0.2)	4.3 (0.2)	4.1 (0.2)
NA	142 (1)	145 (2)	144 (2)	141 (1)	142 (1)	141 (1)
TBIL	1 (0)	0 (0)	1 (0)	0 (0)	1 (1)	0 (0)
TG	1.30 (0.63)	1.73 (1.03)	1.28 (0.53)	1.23 (0.54)	1.33 (0.96)	1.00 (0.40)
TP	57 (4)	57 (3)	56 (2)	55 (4)	57 (5)	55 (4)
UREA	6.4 (0.8)	5.7 (0.8)	6.3 (1.0)	6.0 (1.4)	6.6 (1.2)	5.9 (0.7)

Parameter explanation
ALP – Alkaline Phosphatase IU/L
ALT – Alanine Aminotransferase IU/L
AST – Aspartate Aminotransferase IU/L
CA – Calcium Total mmol/L
CHOL – Cholesterol Total mmol/L
CREA – Creatinine µmol/L
GLDH – Glutamate Dehydrogenase IU/L
GLU – Glucose mmol/L
K – Potassium mmol/L
NA – Sodium mmol/L
TBIL – Bilirubin Total µmol/L
TG – Triglycerides mmol/L
TP – Total Protein g/L
UREA – Urea mmol/L

Discussion

The aim of this study was to evaluate the use of exercise pens as an environmental enrichment for laboratory rabbits. This was done by comparing the behaviour and health of animals that had varying access to exercise including control animals with no access to exercise pens.

Behaviour in cages

The rabbits were inactive (lying or sitting) during the majority of observations in their cages. Rabbits spend about 70 % of their time resting or grooming and about 20 % eating (Stauffacher, 1992), and this corresponds quite well with the observations made in this study. However, according to other studies rabbits in semi-wild conditions may spend up to 70 % of their days searching for food and eating (Trocino & Xiccato, 2006). In an earlier inventory of the behaviour of caged rabbits it was also found that rabbits were inactive during a majority of the day but they also spent less then 10 % of the time eating (Gunn & Morton, 1995). The rabbits of this study had always access to hay which could explain the longer eating time.

There were large individual differences in the behaviour and some animals were lying down during over 80 % of the observations and some almost never was observed moving. Time spent inactive could indicate the state of boredom according to Gunn & Morton (1995). All observations were made during daytime and as rabbits mainly are active at dawn or dusk (Gunn & Morton, 1995; Stauffacher, 1992) the rabbits of this study may appear less active than they were if they had been observed for 24 h. In the study by Gunn & Morton (1995) rabbits were mobile only 1 % of the day. Rabbits in this study moved considerably more even though they were observed during daytime. This could be explained by them living in larger cages than what is normal which gives them a greater opportunity to move in a more normal way. However, limited freedom of movement has been shown to lead to restlessness and stereotypic behaviour (Stauffacher, 1992) and even though these rabbits have more space than normal they are still not able to run and jump freely. This could especially affect the physiological development of the younger rabbits.

Stereotypic behaviour accounted for over 10 % of the behaviour expressed by caged rabbits in the study by Gunn & Morton (1995) and it was most often expressed during the early morning. Expression of such behaviour is probably a sign of frustration and boredom (Morton et al., 1993). Some stereotypic behaviour such as wire-gnawing was observed also in this study but not at all to the same extent. The larger cages and unlimited access to hay in this study probably reduces the amount of these behaviours, but since observations were made during daytime it is probable that it still is more common than what was observed.

During the last weeks when behaviours in cages were recorded the control group spent more time eating than rabbits being exercised. Since their ratio of pellets almost always was eaten shortly after delivery the difference lay in the time spent eating hay. So it seems that when the rabbits are given access to an exercise pen their need for chewing on hay was smaller. This could maybe be explained by them recieving other impressions and not eating for leasure. And during the last week the rabbits exercised three times per week moved more than the controls and those exercised once per week. This could mean that the exercised rabbits are in a better physical condition and have the strength to move around more. It could also indicate that the rabbits which have been exercised often during daytime are more alert during the midday and the other rabbits are more alert around dusk and dawn. Even though this do not mean that the rabbits will get more active of the exercise, at least they will not get tired by it and move less in the cages. Behaviour in cages were affected by the exercise sessions and possibly in a positive way, as the ones being exercised were more active and eat less.

Young rabbits were observed eating more often while the older rabbits had a higher number of recordings for lying. Since the young rabbits were given the same amount of pelleted feed while still growing they need to consume more hay instead which occupies more of their time.

The older rabbits on the other hand have more time doing nothing because of the sparse environment.

Behaviour in exercise pens

The rabbits were active during the majority of observations in their exercise pens and some animals were never observed lying down during a session. This amount of locomotory behaviour is much more than what has been seen in wild rabbits (Stauffacher, 1992). Since it is likely that singly caged rabbits cannot move freely or fullfil their social behavioural needs (Morton et al., 1993) this high degree of movement could be due to some built-up need. It has also been shown that presence of enrichment increases the amount of hopping (Trocino & Xiccato, 2006). The cage environment may induce inactivity and boredom (Gunn & Morton, 1995) and in this new environment the rabbits were able to relieve some of that boredom.

Some specific behaviours with shorter duration were recorded separately. Investigatory behaviour and marking territory was found to be most commonly performed, and they are in a way connected since rabbits often first sniff an area and thereafter marks it. Rearing was also shown often, it is also a behaviour to excert control over the surrounding environment. These three behaviours were performed by all animals at least once. Other less common behaviours were leaping, parading, digging and gnawing. Leaping can be seen as a form of play behaviour and parading is a social behaviour.

The only significant difference found in the behaviour between the rabbits over the entire study period was that the rabbits exercised three times per week had a higher percentage of lying. The difference was seen during week 2 and 4. Rabbits exercised onced per week had a higher percentage of eating and grooming instead during these weeks. Since these differences were found early in the study period and not in the end the rabbits exercised three times per week did not seem to get tired of their new surroundings. They may have gotten exhausted during the beginning of the period but then made use of the exercise area later when in better shape. The rabbits which were exercised once per week were not moving to a higher degree either, but they were eating or grooming more which are maintenance behaviours.There were also minor differences during the first week but these were probably individual and not due to the different amount of exercise since the study barely had begun.

Older rabbits had a higher percentage of grooming and lying, whereas younger rabbits had a higher number of recordings for leaping and rearing in their pens. The younger rabbits may need more space to be able to play, and the different locomotory activities are important for their physiological development (Morton, et al., 1993).

As rabbits are highly social animals (Trocino & Xiccato, 2006) and the effects of social deprivation unknown (Gunn & Morton, 1995) it is recommended to house rabbits singly only if necessary (Morton et al., 1993). Rabbits in groups have a better quality of life (Verga et al., 2007) and show no stereotypic behaviour (Podberscek et al., 1991). Singly caged animals should have visual and olfactory contact with conspecifics (Nevalainen et al., 2007; Verga et al., 2007). This can only be met to some degree in the ordinary cages. The exercise session enabled the rabbits to interact with conspecifics even though they did not have direct contact. In addition to seeing other rabbits in the pens alongside they could also smell the markings made by rabbits exercised earlier. Rabbits held in groups spend less time feeding and resting and more time exploring the environment (Podberscek et al., 1991), and this corresponds with the results of this study as the rabbits exercised spent more time moving and less time lying and eating.

Placement in cages or pens

A majority of the time in the cages was spent on the floor. Individual differences were huge and while some rabbits were on the shelf during a majority of their observations others never used the shelf. The rabbits generally spent more time on the shelf than under it and this was most obvious in the control group. There were no major differences in the use of the shelf between rabbits being exercised and rabbits in the control group.

In the exercise pen rabbits also spent a majority of the observations on the floor, but there were large individual differences and some rabbits were on the floor during 10 % of the observations while others were there for 90 %. However, most rabbits used all the available space. The shelf was slightly less popular than in the cages and the only difference between the two groups was that rabbits exercised three times per week spent almost twice as much time on top of the shelves. They may have become more used to the pen environment and felt safer to expose themselves on top of the shelf. But this difference was not significant, probably due to the large individual differences. It is important that the rabbits are able to hide (Morton et al., 1993), but maybe the shelf did not offer this because of the lack of a solid wall in the back and on the sides. Perhaps the shelf had been used more for hiding if it had been more enclosed.

The box with wood shavings and hay which was a new enrichment to their environment was very much used, more than the shelf. Also here there were huge individual differences and while some rabbits almost never used the box others spent almost 90 % of the observations in it. All rabbits visited the box at least once. Rabbits exercised once per week spent almost twice as much time in the box as those exercised more frequently but this difference was not significant, probably due to the large individual differences. The box offers an opportunity for digging and rabbits exercised less frequently may have a larger need to express that behaviour than those who have access to the box more often. The effect of a lack of opportunity for digging is unknown (Gunn & Morton, 1995).

Environmental enrichments in the cage or pen

The chewing stick was only used by one rabbit, but the gnawing on a stick was performed between recordings. All recorded gnawing is performed on other parts of the pen. The chewing stick was not popular in the cages either. In another study over 90 % of the rabbits regularly gnawed on pine-wood sticks (Stauffacher, 1992). The chewing stick in this study was made of aspen, maybe the rabbits would appreciate another sort of wood more. In another study by Lidfors (1997) rabbits only rarely interactes with gnawing sticks made out of aspen and the access to sticks did not have an effect on the amount of abnormal behaviours expressed. The rabbits in this study also had an unlimited access to hay so maybe their need for gnawing on sticks was reduced by that possibility. Branches have been proposed as an enrichment as it can offer both opportunities to gnaw and chinmark (Morton et al., 1992). They are also more different in appearence, rabbits in this study has unlimited access to their chewing stick and it offers no new stimuli and the rabbits might get bored with it (Morton et al., 1993; Verga et al., 2007). However, all enrichments has to be autoclaved before entering the animal unit, and branches may not be so easy to autoclave.

The plastic ball was maximally interacted with 3 times by one rabbit and half of the rabbits never interacted with it. The low interaction grade could be because the ball was not optimally designed, it was quite heavy and had only a few holes to grab. But it may also be that playing with a ball is not such an important behaviour to perform and other things were more appealing in the exercise pen.

Behaviour in cages during exercise sessions

The behaviour performed in cages seemed to be affected by other rabbits being exercised. An increase could be seen in behaviours such as moving and other active behaviours such as digging, rearing and gnawing. Lying and sitting on the other hand decreased during times when other rabbits were being exercised. Presence of enrichment has been shown to increase hopping (Trocino & Xiccato, 2006) and seeing other rabbits in this way could possibly be regarded as a form of enrichment. But it has also been shown to reduce gnawing of the cage (Trocino & Xicacto, 2006) which in this study instead increased. Since the rabbits became generally more active when others were being exercised it could result in an increase of gnawing the cage instead as stereotypic behaviour is most frequent when rabbits are more active (Gunn & Morton, 1995).

Even though rabbits in all groups seemed to be affected by seeing other rabbits being exercised they may not be affected to the same degree. Rabbits that had been exercised themselves had a much higher increase of moving than rabbits in the control during times when other rabbits were being exercised. Perhaps these rabbits expected to be exercised soon or they were simply activated by the presence of this new form of enrichment, as new enrichments has been shown to increase play behaviour (Wood-Gush & Vestergaard, 1991).

Stress and general health

Hiding behaviour was never recorded in the cages, since it is is a familiar environment and there were no disturbances during the observations this could be expected. In the pens hiding occupied around 0.5-1% of the observations. Almost all of this behaviour occurred during the first exercise session. Hiding behaviour was only shown by some rabbits. Since the pens were a new environment it is not strange that some rabbits may become stressed in the beginning. Since the percentage of this behaviour then dropped it is probable that the rabbits got accustomed to the new surroundings and no longer became stressed.

This assumption is further supported by the results of the corticosterone levels as they were significantly elevated after exercise only during the first week for rabbits being exercised once per week. Corticosterone levels are a good indication of the stress response; it is sensitive to social stress due to eg. competition (Verga et al, 2007) and levels are likely to increase by fear (Morton et al., 1993). Since no elevation could be detected in the later weeks the rabbits were probably no longer stressed by the environment and the proximity of other males. No adverse health effects could be detected in the general health parameters either.

Young rabbits gained weight during the study period while older rabbits lost weight. As rabbits exercised lost more weight than those in the control group it is likely that the exercise had a positive effect on the weight of the rabbits even though the differences did not reach significance due to the small groups. It has been found before that group penned rabbits suffers from obesity in a lower degree due to a greater opportunity for exercise (Morton et al., 1993).

The use of an exercise pen instead of a change in the rabbit’s cage leads to an increase in the handling of the rabbits as they will have to be moved to the pen and back each session. Frequent handling of the rabbits is important and gives more docile animals (Morton et al., 1993).

Conclusions

The exercise pen offers a larger area to explore and an opportunity to play. The rabbits appeared less bored in their pens and were more active in them, and there they could perform locomotory behaviours such as leaping and running. As exercise pens allow a broader range of natural behaviours to be expressed it gives a psychological improvement. There were no significant differences between rabbits exercised once or three times per week and the behaviours expressed in the pens did not change with time. Rabbits in cages also seemed more active while others were being exercised. Signs of improvement in their physical health could be seen as they lost weight and no signs of stress could be measured when they had got accustomed to the new environment.

Further research and developments

I believe more research is needed for constructing an optimal pen with the best possible enrichment objects. The length of the pen is important to allow the rabbits to run freely, the measurements of the pens in this study seemed adequate to fullfil this. It is also important with safe pens and besides the rubber mat which prevents slipping it is significant that the rabbits can not hurt each other or escape. As rabbits only should be caged singly if needed, the exercise session could also give the rabbits an opportunity to interact with conspecifics if the pens are designed to enable visual and olfactory contact. Enrichment objects may be rotated in both the cages and pens to prevent the rabbits getting bored with them.

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