Chapter 10

THE WELFARE OF LABORATORY RABBITS

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1. INTRODUCTION

Rabbits were the fifth most commonly used mammalian laboratory animal after mice, rats, guinea pigs and pigs in Sweden during 2002 (CFN 2003). According to the latest statistics for the EU member states, 227 366 rabbits were used during 1999 (Commission of the European Communities 2003). Both domesticated rabbits and European wild rabbits may be used for experimental research, but there are several problems in keeping and breeding the European wild rabbit (Bell 1999). Today the most common breeds used are the New Zealand White (NZW), the Dutch and the Half Lop (Batchelor 1999). Most laboratories buy these breeds as health defined (previously called Specific Pathogen Free) from accredited breeders (Townsend 1969, Eveleigh et al. 1984).

Rabbits are used for many different purposes with a large number being used for antibody production, but also for orthopaedics and biomaterials (Batchelor 1999). The rabbit is especially suitable for studies on reproduction (Batchelor 1999). Rabbits are also used for cardiac surgery, and studies of hypertension, infectious diseases, virology, embryology, toxicology, experimental teratology (Hartman 1974), arteriosclerosis (Clarkson et al. 1974) and serological genetics (Cohen and Tissot 1974).

Laboratory rabbits are by tradition kept individually in small cages with restricted food availability. This has led to several physiological problems related to the fact that they move too little, as well as behavioural disorders. Over the past 10-15 years many laboratories have improved the housing for

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rabbits, both in cages and also by introducing floor pens and group housing. However, there are still some aspects to be addressed in rabbit housing especially in relation to the fact that they can be very aggressive when kept in groups.

The aim of this chapter is to present the most recent knowledge about the laboratory rabbit's biology, behavioural needs, optimal environment, housing, feeding, care, handling, health and experimental techniques, in order to ensure their optimum welfare.

2. BIOLOGY AND BEHAVIOURAL NEEDS

2.1 Domestication

The laboratory rabbit originated from the European wild rabbit (*Oryctolagus cuniculus*, Figure 10-1) (Harcourt-Brown 2002). Rabbits were already kept in fenced hunting areas by the Romans 2000 years ago, and domestication appears to have started around the year 500 AD by the French. European wild rabbits were introduced to Great Britain by the Normans soon after the Conquest of 1066 (Meredith 2000). The domestication most probably occurred in the Monasteries, where several different breeds were developed.



Figure 10-1. European wild rabbits in a fenced grass area in England used for behavioural ecology research (photograph by Lena Lidfors 1995)

During the first half of the 20th century many new rabbit breeds were developed, and today there are 76 recognized breeds of fancy fur and Rex rabbits (British Rabbit Council 1991). The New Zealand White (NZW) was accepted as a breed in 1925 according to American Standards (Batchelor 1999). This rabbit has been bred for fast growth for meat production and their white pelts which can be dyed any colour, but it has also become a very commonly used laboratory breed. The negative impact of this breeding emphasis is that laboratory New Zealand White rabbits relatively easily become obese, and food is often restricted in singly housed animals to prevent this. Other rabbit breeds more suitable for laboratory research have been developed more recently, taking into account the purpose of a certain research area, for example the Half Lop, which has long ears suitable for repeated blood samples during anti-body production.

2.2 General biology

The natural diet of rabbits consists of grass and herbs, but also fruit, roots, leaves and bark (Cheeke 1987). The rabbit needs coarse fibre, not just grass (Brooks 1997, Meredith 2000). The rabbit is caecophagous (often called coprophagous), i.e. it needs to ingest the smaller, soft and green coated faeces pellets produced by the caecum 4-8 hours after feed intake in order to survive (Brooks 1997). The rabbit takes the soft faecal pellets directly from its anus, whereas the harder pellets are placed on specific latrines close to the territory borders (Donnelly 1997). The rabbit has a light and fragile skeleton that only makes up 7-8 % of the body weight (Donnelly 1997).

Rabbits can adjust to different ambient temperatures, but have problems with too high temperatures and therefore seek shade from sun light in their burrows or under bushes. They have a 190° field of vision, but can not see the area beneath their mouth. They use their whiskers and the sensitivity of the lips as well as scent and taste during foraging (Meredith 2000). The whiskers are also used during orientation in the burrows and dens. Scent and taste is more important in identifying members of the own breeding group than vision. They have good hearing, and their big ears occupy about 12 % of the total body area (Meredith 2000).

Reproduction in the European wild rabbit is seasonal and appears to be determined by the interaction of a number of environmental factors, for example daylength, climate, nutrition, population density and social status (Bell and Webb 1991, Bell 1999). Sexual maturity occurs at the age of 5-7 months in males and 9-12 months in females, depending on climate (Myers et al. 1994). Domestic rabbits are sexually mature at 4-6 months of age (Falkmer and Waller 1984), but the small breeds are sexually mature earlier

than the medium and large breeds (Bennet 2001). Before mating the rabbits circle around each other, parade side by side, jump over each other and sniff around the genital region (Lehmann 1991). Hafez (1960) found seven degrees of sex drive which ranged from aggressive with immediate mounting and ejaculation to offensive reaction with general smelling of the skin, biting and no ejaculation. The buck mating takes only seconds, after which he dismounts to the side or backwards (Bennett 2001). When allowing mating the doe raises the hind quarters. The ovulation is induced by the mating and the sperm is waiting until the egg appears after 8-10 hours, when conception takes place (Bennett 2001). The doe can mate immediately following parturition, and if the young are removed after delivery she will be sexually receptive for at least 36 days (Hagen 1974).

The doe is pregnant for between 28-30 days (Bell 1999) and 30-32 days (Batchelor 1999). Some days before the birth the doe digs an underground nest either within the main warren or in a separate breeding "stop" dug specifically for the purpose (Bell 1999). These are short, shallow burrows dug at a distance of 10-50 yards from the warren. High ranking females have been found to often give birth to their young in a special breeding chamber dug as an extension to the warren, whereas some of the subordinate females are chased away from a warren and forced to drop their litters in isolated breeding "stops" (Mykytowycz 1968). The survival rate is much higher for the high ranking than the low ranking females. The doe collects and carries grass to her burrow and shortly before giving birth she plucks her own fur from the belly, sides and dewlap, and places it on top of the grass (Gonzáles-Mariscal et al. 1994, Bell 1999). The better the construction of the nest the higher the survival rate of the young (Canali et al. 1991).

The rabbit foetuses are born with no or only little hair cover, and are deaf and blind (Batchelor 1999). They each weigh around 50 g at birth and gain about 30 g per day (Falkmer and Waller 1984). The size of the litter is 3-6 young for European wild rabbits, and 4-12 young for domesticated rabbits (Batchelor 1999). At birth the young rabbits are very sensitive to cold, but as their fur grows out within a couple of days they become less sensitive (Batchelor 1999). The eyes open at 10-11 days (Kersten et al. 1989, Batchelor 1999), or 9-12 days (Falkmer and Waller 1984). Hearing develops at the same time, but no reference on this has been found. When the young are born the doe leaves the burrow and covers the entrance with soil, urine marks it, and then leaves (Mykytowycz 1968). She returns to the burrow once a day, and then digs herself into the burrow and nurses her young (Bell 1999). In a study on Dutch Belted rabbits the nursing took place in the early morning and lasted for 2.7-4.5 minutes (Zarrow et al. 1965). When given the opportunity to retrieve their young, does did not perform this behaviour (Ross et al. 1959). The doe closes the burrow and or breeding "stop" for

about 21 days, after which the young emerges onto the surface (Bell 1999). The young are very mobile at four weeks of age, and leave their breeding stop soon after emergence (Lloyd and McCowan 1968). At that time they start seeking forage, but they continue to suckle for some more weeks. The does reach maximum milk production two weeks after giving birth, this declines during the fourth week and they may lactate for an additional 2-4 weeks. At 8 weeks of age the young are consuming approximately 90% of their intake in the form of plant proteins (Hagen 1974). In commercial rabbit breeding the young are usually weaned and separated from the doe at 6-7 weeks of age (Hagen 1974, Bennett 2001).

NZW does in semi-natural enclosures behave in a similar way towards their young as the wild rabbit, but when the young are about 18 days old their mother no longer closes the entrance to the breeding stop properly and the young are nursed outside (Lehmann 1989). The mother-young relationship was quite loose, and the mothers were not preferred social partners for the young except for suckling attempts. Nursing was unlikely after four weeks when the doe littered again within a few days, but suckling attempts occurred up to 60 days (Lehmann 1989).

2.3 Natural behaviour

The behaviour of the European wild rabbit (Bell 1999, Figure 10-1) and the behaviour of NZW rabbits kept in a free-range enclosure have been studied (Lehmann 1991, Stauffacher 1991). Studies of natural, free-ranging and enclosed populations in Australia, New Zealand and United Kingdom have shown that European wild rabbits live in small, stable, territorial breeding groups (Parer 1977, Gibb et al. 1978, Cowan 1987, Bell and Webb 1991). The breeding group is generally described to defend its territory, a core area with a warren within a larger home range, by patrolling the borders and scent-marking (Bell 1999). However, in some studies it has been found that breeding groups only occupy single warrens (Myers and Schneider 1964, Bell 1977), whereas in other studies of free-living populations of rabbits most breeding groups used multiple warrens (Dunsmore 1974, Parer 1977, Wood 1980, Daly 1981, Cowan 1987). During the peak feeding periods at dawn and dusk members of neighbouring breeding groups may move out from their territories to forage in communal grazing areas (Bell 1980). The number of entrances per warren was found to be 11.5 ± 8.0 SD, with a range of 1-37 (Cowan 1987). The different breeding groups together make colonies of up to 70 rabbits. The social unit is the breeding group, which consists of 1-4 males and 1-9 females (Meredith 2000).

Within breeding groups separate, stable, linear dominance hierarchies are formed within each sex (Bell 1983). The strict rank order is maintained by

rabbits keeping a fixed distance from one another and exhibiting submissive behaviour. Early studies of the European wild rabbit in Australia revealed that a substantial portion of the rabbits' daily activity was filled with direct or indirect aggression (Mykytowycz and Rowley 1958, Mykytowycz and Fullager 1973). It was concluded that this behaviour underlies the social and territorial organisation, which are the main factors affecting the numbers of free-living populations of this species (Mykytowycz 1960). In some studies does have been found to be more aggressive than bucks (Southern 1948, Myers and Poole 1961), or found to fight as strongly as males (Lockley 1961). Females mainly fight over breeding burrows (Cowan and Garson 1985), and are more aggressive towards juvenile females (91% of interactions) than juvenile males (Cowan 1987). Males are tolerant to females, young and sub-adults in their breeding group, and have even been observed to interrupt aggressive interactions between females. When the number of rabbits living together increased, fighting increased dramatically (Myers 1966). In nature young males normally move to a new social group before the start of their first breeding season, while young females stay on to breed in their breeding group (Parer 1982, Webb et al. 1995).

Lehmann (1991) has described 20 different social behaviours of New Zealand White rabbits kept in a semi-natural enclosure, and how the social behaviours change with age. Indifferent contacts were body-to-body, nose-to-body, nose-to-nose contacts and anogenital nuzzling. Amicable behaviour comprised cuddling up and allogrooming. Subdominant behaviour was crouching, retreating and fleeing, whereas aggressive behaviour was circling, nudging, attacking and chasing. Actual fights with aggressive leaping and ripping were observed only 7 times. Mykytowycz and Hesterman (1975) made paired encounters between male and female European wild rabbits and New Zealand White rabbits in a home pen, and found that aggression was equally prevalent in both sexes and inter-sexual fighting occurred just as frequently as fighting between members of the same sex. The domesticated NZW rabbits were fighting just as much as the wild rabbits, but paired domestic females and domestic males paired with wild rabbits of both sexes fought less frequently and less viciously than other paired rabbits.

Rabbits have three specialised scent glands, i.e. in the anal region, in the groin and under the chin (Mykytowycz 1968). The territory of rabbits is scent marked by placing faeces in dunghills, and about 30 such dunghills have been located in a typical warren. The rabbit also marks its territory by pressing the under-chin against structures in its environment so that droplets from its sub-mandibular glands are forced through pores of the skin. Scent marking is more intensive by males than females and more in dominant than sub-dominant individuals, and it is correlated with larger anal and sub-mandibular glands in dominant males (Mykytowycz 1968). The most

dominant animals of a warren were found to possess the heaviest anal glands. Males scent mark females and young rabbits of their breeding group by spraying urine on them. Females scent mark their young, attack other young within the same breeding group, and may chase and even kill young from other breeding groups. Females may attack even their own young if they have been smeared with foreign urine (Mykytowycz 1968).

The rabbit has a home-range for performing foraging behaviour. The size of the home-range varies depending on food availability, age of the rabbit, the rabbit's status within the breeding group and number of rabbits in the group (Donnelly 1997). The home-range of wild European rabbits has been estimated to be 5 ha (Myers et al. 1994), 0.4-2.0 ha (Cowan and Bell 1986), or 8 ha (Vastrade 1987). Cowan (1987) found that that the mean asymptotic male range was 0.71 ha and female range 0.44 ha.

The rabbit is mainly a nocturnal animal, emerging from the burrows in late afternoon (Fraser 1992). The old bucks emerge first, about four hours before sunset (Mykytowycz and Rowley 1958), and by sunset 90% of the rabbits have emerged (Fraser 1992). The rabbits are visible on the ground during 11-14 hours of the diurnal cycle (Mykytowycz and Rowley 1958). When the rabbits are above ground they spend about 44% of their time eating, 33% inactive, 13% moving and 10% on other activities (Gibb 1993). During semi-natural conditions young NZW rabbits were active for an average of 30% of the daytime. Feeding on pellets and grazing took a third each of the active time, and the remaining third was spent exploring, gnawing, intensive locomotion and, for the older rabbits, engaging in sexual behaviour (Lehmann 1989). The rabbit's choice of habitat depends on the opportunities to find shelter and protection, and where the soil is loose it digs burrows, and where the soil is more compact it seeks protection in dense vegetation (Kolb 1994). In case of danger a rabbit can stamp with its hind feet, thus causing the other rabbits to flee underground (Black and Vanderwolf 1969). If it is too late to flee the rabbit can freeze, i.e. stop in its movement and be completely motionless. A rabbit can, if caught by a predator, emit a high distress scream (Cowan and Bell 1986), which may cause the predator to release its prey. Apart from this and some low sounds during mating and mother-young care rabbits are silent animals.

The movement patterns of rabbits consist of hopping, crawling and intensive locomotion, i.e. running, start-and-stop, jumping, double and capriole (Kraft 1979, Lehmann 1989). Hopping is usually performed when rabbits move over longer distances, whereas crawling is performed when feeding on grass or exploring on the spot and during social encounters (Lehmann 1989). Rabbits perform comfort behaviours such as licking and scratching themselves, shaking the body, rubbing against objects and stretching their body.

Several studies have compared the behaviour of wild European rabbits with those of domesticated strains of rabbits (reviewed by Bell 1984). Both wild rabbits and domestic strains have reproductive seasonality, suckle their young only once every 24 h, show two main feeding periods at dawn and dusk, form breeding groups with separate linear dominance hierarchies among male and female members, and reproduce successfully with the female digging breeding burrows, building nests for their young and covering the entrance to the burrows with soil (Bell 1984). The difference found is that domestic rabbits rest more above ground during the day (Stodart and Myers 1964) and that males chin-mark more often (Kraft 1979) also in unfamiliar territory (Mykytowycz 1968). New Zealand White females had fewer days in anoestrus than wild rabbits, and therefore produced more litters with a larger mean size (Stodart and Myers 1964). When European wild rabbits were brought into the laboratory they failed to breed, and females born into the laboratory as a result of egg transfer from wild to domestic mothers retained their nervous disposition and failed to mature sexually (Adams 1987).

2.4 Abnormal behaviour

When rabbits are kept alone in traditional small cages several abnormal behaviours may arise. The most obvious abnormal behaviour is stereotypy, e.g. wire-gnawing, excessive wall-pawing (Lehmann and Wieser 1985, Wieser 1986, Bigler and Lehmann 1991, Loeffler et al. 1991, Stauffacher 1992). When many rabbits gnaw on the wire of their cages there might be a high sound level in the animal room (Figure 10-2). Digging may be constrained by the solid floor of the cage (Podberscek et al. 1991). The stereotypic behaviour substitutes for natural behaviours which cannot be performed in standard laboratory housing (Stauffacher 1992) and may indicate frustration, anxiety or boredom, and develop in stages involving a progressive narrowing of the behavioural repertoire (Gunn 1994). Individually caged rabbits show stereotypies, such as somersaulting, no full hops, less activity than group-penned rabbits, and less marking and investigatory behaviour than in group pens (Podberscek et al. 1991). Social isolation can induce the physiological symptoms of stress, which are relieved by the presence of conspecifics (Held et al. 1995).

There are indicators of boredom in rabbits such as hunched posture (Gunn and Morton 1995), inertia (Metz 1984), and a staring coat and dull eyes (Wallace et al. 1990). Prolonged inactivity associated with unresponsiveness, may, like stereotypies, be associated with brain chemistry changes which make the problem seem less bad (Broom 1988). Gunn and Morton (1995) mention problems with under-grooming which may lead to a staring coat, and over-grooming which may lead to hair-balls which in turn may cause intestinal stasis (Jackson 1991) and lead to death by gastric trichobezoars (Wagner et al. 1974). Under-eating, causing weight loss and over-eating causing obesity are other behavioural problems (Gunn and Morton 1995).



Figure 10-2. A male New Zealand White rabbit gnawing on the wire of its cage (photograph by Lena Lidfors 1994).

The very limited freedom of movement in caged rabbits leads to changes in locomotion which prevent hopping, thus causing changes in the locomotion apparatus (Lehmann 1989, Stauffacher 1992). It is mainly in the femur proximalis and the vertebral column that changes of the bone structure occur, and the changes consist of thinner and less strong hollow bones (Lehmann 1989, Drescher and Loeffler 1991a,b). Lehmann (1989) showed that growing rabbits perform almost no hopping and intensive locomotion mainly during playing when kept in cages compared to an outdoor enclosure. Crawling, which was the most common movement pattern, was slightly less common in the cages. These rabbits often performed interrupted jumps, where the hindlegs only were lifted slightly and then put down again, thus not being used as in normal hopping. Abnormal postures may also be shown due to spatial restriction for lying stretched out during resting or performing stretching behaviour (Gunn 1994).

Rabbits may show restlessness, e.g. afunctional bouts of activity with disconnected elements of feeding, comfort, resting, alertness and withdrawal behaviour alternating with locomotion, which leads to space-time organization disorder as well as panic (Lehmann and Wieser 1985, Bigler and Lehmann 1991, Stauffacher 1992).

An abnormal behaviour related to reproduction is disturbed sexual behaviour which leads to low conception rates (30-70 %) (Stauffacher 1992).

One reason for this may be that for mating the doe is placed in the bucks' cage a few days to several weeks after she last gave birth. The mating performed then has been described more as a rape than normal mating behaviour (Stauffacher 1992). However, the doe may be aggressive against the buck if he is placed in her cage (Bennett 2001). Around giving birth the doe may show disturbed nesting behaviour and nesting stereotypies which may lead to rearing losses (Wieser 1986, Wullschleger 1987, Loeffler et al. 1991). The doe may also show disturbed nursing and cannibalism caused by restlessness of the mother and her pups which may also lead to rearing losses (Bigler 1986, Brummer 1986, Stauffacher 1992).

2.5 Behavioural needs

Based on the previously presented research on the abnormal behaviours performed by housed rabbits, the behaviour of Wild European rabbits and free-ranging New Zealand White rabbits many researchers involved in this area have had an insight to the behavioural needs of rabbits. Based on this research and on several discussions in international workshops suggestions have been made on the following behavioural needs and behaviours involved in performing them:

- 1) Locomotion and exercise: crawling, hopping, running, jumping, quick changes of direction.
- 2) Control, "security and safety": withdrawal, digging, burrowing, sit upright, rearing, sniffing, "stamping".
- 3) Foraging: eating, drinking, caecophagi, gnawing, manipulation of resources, searching, exploration, exploitation of resources.
- 4) Behavioural rhythm: extended periods of rest and grooming, activity.
- 5) Companionship and social contact: different social behaviours.
- 6) Demonstrate presence; scent marking by anal and submandibular glands.
- 7) Female choice in mating; circling, parading, jump over and sniff genital region before mating.
- 8) Nest and burrow with constant temperature: digging, carrying nesting material, plucking fur.
- 9) Separate from litter: nursing once/twice a day, close nest entrance.
- 10) Warmth and protection: huddle in hair-nest for new-born rabbits.
- 11) Learn how to cope: play and exploration in young rabbits.

As some of the suggested needs lead to both physical and behavioural problems if they are not performed, e.g. foraging, movements, other are more questionable, for example digging. There is a need for more research to establish exactly which behavioural needs are so important that one must provide them in the rabbit housing, i.e. essential needs.

3. OPTIMAL ENVIRONMENT

3.1 Light and noise

Laboratory rabbits are recommended to be kept in animal rooms with a regular light:dark cycle and isolated from external lighting fluctuations (Batchelor 1999). Some laboratory facilities have introduced artificial dawn and dusk periods, usually working for 30 minutes before full light comes on in the morning and 30 minutes before the light is shut down in the evening. However, there is still some debate as to the relative merit of creating an artificial dawn and dusk period (Batchelor 1999).

As rabbits are more nocturnal than diurnal a partially reversed lighting schedule can be established to observe their activity (Batchelor 1999), but rabbits may also switch to a more diurnal pattern of activity due to noise or scheduled feeding in the laboratory (Jilge 1991).

The optimal light intensity in rabbit rooms is recommended to be 200 lux at one metre above the floor (Iwarsson et al. 1994). Too high levels of illumination can result in retinal degeneration in some albino mammals, and this may include NZW rabbits (Batchelor 1999). Solid side caging will reduce the amount of light, and where in the rack a cage is situated may also have an impact on light intensity. If rabbits are housed in floor pens, shelves and boxes may provide hiding places also from high light intensity.

The rabbits hearing range thresh-hold level is 75-50 000 hertz with the best hearing at 2 000 to about 9 000 hertz (Iwarsson et al. 1994). The rabbit is sensitive to high sound frequencies (Milligan et al. 1993), but also sudden noise may scare rabbits and lead to injures. Background music masks sudden loud sounds, and is claimed by some to result in lower excitability (Batchelor 1999).

3.2 Temperature and humidity

The mean room temperature for rabbits is recommended to be 18 °C with a range of 15-22 °C (GV-SOLAS 1988). The Lower Critical Temperature is -7 °C and the Higher Critical Temperature is 28.3 °C (Spector 1956). Rabbits only have sweat glands on their lips, and lower capacity to ventilate through their mouth than dogs (Donnelly 1997). Wild rabbits avoid high temperature and stay away from direct sunshine, and during the day they stay in the cooler burrows. The ears of the rabbit are highly vascular and function as ventilators during high ambient temperature. In the rabbit room a humidity level of 55 ± 10 % is recommended (Batchelor 1999).

3.3 Ventilation and hygiene

In the animal room a ventilation rate of 15-20 air changes per hour is recommended (Iwarsson et al. 1994). Air changes lower than this may be satisfactory if the cleaning routines is of high standard and the stocking density is low (Batchelor 1999).

Ammonia level should never exceed 10 ppm. When measuring ammonia level in a modified farm animal building with group housed rabbits the highest level was 1-2 ppm (Batchelor 1999). High ammonium levels can inactivate the cilia in the air ways of rabbits. High levels of CO_2 may become a problem for the rabbits if the ventilation is not working properly.

Due to normal changes of the fur 2-3 times per year rabbits loose hair in the laboratory unit. The hair may fly around in the animal room, and eventually ends on the floor. Especially during handling of the rabbits hair may be released.

Rabbits kept in cages should be moved to a clean cage once a week, but the waste pan under the perforated floor could be changed more often. If rabbits are kept on wire net floor and a band can be moved under the cages this should be cleaned once per day. Many laboratories today have dishwashers where the whole rack is washed in warm water with disinfectants and dried at high temperatures thus working as an autoclave. The feed is usually pseudo-pasteurized (70 °C for 10 minutes) at the producers. In order to reduce the risk of bringing pathogens into the laboratory, hay and any environmental enrichment should be autoclaved or irradiated with cobalt, but the latter is not so common.

Rabbits kept in floor pens should be taken out of the pen after which the pens should be thoroughly cleaned and disinfected. Batchelor (1999) suggest that this should be done at least once per month. However, the laboratories we know of carry out this cleaning at least once per week and more often when needed in order to maintain a high level of hygiene. If cleaning is not done often the rabbits can become infected with coccidiosis. Cleaning changes the olfactory environment for the territorial rabbits and may actually be stressful for them (Batchelor 1999).

3.4 Transport

The rabbits should be in good condition before transport. Usually rabbits are transported in containers made of strong cardboard, fibreboard, fibreglass or

wood with wire mesh windows for ventilation, and equipped with a filter in summer time (Swallow 1999, Batchelor 1999). The floor of the container should be waterproof and covered with absorbent material, for example saw dust. For more detailed information about the general requirements and species requirements when transporting animals see Swallow (1999).

Regulations for transport of animals may differ for the EU (according to the Convention of Europe) and for different countries over the world. In England and Sweden, when transporting rabbits of less than 2.5 kg the smallest area per animal should be 1000 cm² in non-filtered crates (winter) and 2 000 cm² in filtered crates (summer) (Swallow 1999, SJVFS 2000:133). In Sweden minimum container height should be 24 cm, and in England it should be 21 cm. In the Guide to the Care and Use of Experimental Animals from the Canadian Council of Animal Care (Olfert et al. 1993) it is stated that the rabbits should be transported in disposable containers with sufficient space to allow the animals to stand, lie down and turn around. Transporting rabbits in compatible pairs, for example siblings, has been practised by a breeder in Sweden with good results.

The transportation vehicles should be equipped with thermometers and ventilation that can cool down the air during warm weather and provide heating during cold weather. A source of water, for example a gel, and feed, for example a carrot, an apple or straw, sufficient for the duration of the transportation has to be provided (Batchelor 1999, Olfert et al. 1993). Swallow (1999) suggests that rabbits should not require feeding for journeys of less than 24 h duration, but if unforeseen delays occur rabbits could be given carrots, fruit, hay or grain.

After arrival at the new animal housing the rabbits should be checked for any health problems and injuries, which could either be caused by the transport or which may have been acquired before the transport. The rabbits should be given 1-2 weeks of acclimatization time after the transport before they are used for research. This will depend on the degree of stress the animals were under at arrival, which in turn may depend on the nature of the journey, its duration and which parameter is being assessed during the recovery period (Swallow 1999). Olfert et al. (1993) state that newly acquired rabbits should be held in guarantine for at least three weeks, and examined regularly for disease. Animals dying during this period should be subjected to complete post-mortem examinations. Most laboratories we have contact with bring rabbits from suppliers of health defined rabbits bred behind a barrier. Often most of the rabbits come from the same supplier, thus there is no need for quarantine. However, when purchasing rabbits from different suppliers we recommend placing them in different animal rooms at least during an acclimatization period of up to three weeks.

There has been very limited research on the effect of transport on rabbits. Batchelor (1999) found considerable differences in body weight in rabbits that were housed in group pens compared to solitary in cages after transportation. The loss of weight in rabbits after transport is mostly due to a loss of gastrointestinal contents in of about 10% of the total body weight (Swallow 1999). These losses are probably maximal after about 15 h of transport, and similar to depriving animals of food and water during the same amount of time. However, the loss in live weight can take up to 7 days to recover (Swallow 1999). Our own research on the effect of providing male rabbits with or without hay and with a change or no change in the feed after a 10 h transport by truck, plane and truck showed that providing hay had a significant effect of reducing the occurrence of diarrhoea (Lidfors pers. com.).

4. HOUSING AND FEEDING

4.1 Housing in cages

Laboratory rabbits are traditionally housed singly in barren cages over periods of several weeks up to several years, depending on the research purpose. The cages for laboratory rabbits have changed from traditional wooden hutches to galvanized iron, aluminium, stainless steel and now plastic caging (Morton et al., 1993). Today three main types of cages can be found; wire, sheet metal with wire front and plastic with wire front (Stauffacher et al. 1994). Housing in small barren cages can lead to several abnormal behaviours and reduced welfare of rabbits (see section on abnormal behaviours). The cages with solid sides, back and top tend to isolate the animals, and prevent them from seeing the source of disturbance. This may cause the rabbits to be jumpy, and in breeding units lead to higher losses due to cannibalism. In order to allow rabbits to see their surroundings it is recommended that barred "windows" occupy 30-50 % of the total wall area (Stauffacher et al. 1994).

In order to allow rabbits to perform normal hopping movements and sit upright the regulations in several countries (Switzerland, United Kingdom) require that cages for rabbits are much bigger than the recommendations from the European Union and the World Rabbit Science Association (Table 10-1). The heights of the cages should be from 40 cm for rabbits <2 kg up to 60 cm for rabbits >5 kg in the Swiss Ordinance of Animal Protection 1991 (Stauffacher et al. 1994). In the suggestions for new rules in the Appendix A from the Council of Europe it is written that cages and pens for rabbits >10 weeks of age should be 3 500 cm² (45 cm height) for <3 kg rabbit, 4 200 cm² (45 cm height) for 3-5 kg rabbit and 5 400 cm² (60 cm height) for >5 kg rabbit. The weights are for the final body weight that any rabbit will reach in the housing. The floor area is minimum floor area for one or two socially harmonious animals, and the height is a minimum height. It is also stated that a raised area, i.e. shelf, must be provided within the cage, and if not providing a shelf the cage size must be 33% larger for a single rabbit and 60% larger for two rabbits. The suggested new rules in Sweden have been agreed since 1995, but so far no decision has been taken by the Agricultural Board.

	Swiss Ordinance	UK Home	European Union	World Rabbit
Weight (kg)	of Animal	Office 1989	Council	Science Assoc.
	Protection 1991		Directive 1986	1992
< 2	3400	4000	1400	2000
2	4800	4000	2000	2000
3	-	4000	2500	-
3.5	7200	-	-	-
4	-	5400	3000	3000
> 5	9300	-	-	-
> 5.5	-	-	-	4000
> 6	-	6000	-	-

Table 10-1. The recommendations by different authorities for minimum cage area (cm²) for rabbits

During the last 10 years there has been a development of new cage systems (Figure 10-3), using a cage with an increased floor area, a higher cage for upright sitting, a shelf for rabbits to hop up onto or hide under, racks to make hay feeding easier and flexible cage racks so that several cages can be built together. A raised shelf reduces restlessness, grooming, bargnawing, and timidity of being captured (Berthelsen and Hansen 1999). Wild rabbits rear when they are looking at their surroundings (Lockley 1961), and caged rabbits use boxes and shelves provided as sources of enrichment and lockout posts (Hansen and Berthelsen 2000). Mirrors as a form of enrichment do not provide an image of a companion, but probably stimulate activity by enriching the visual environment and increasing the amount of movement perceived by the rabbit (Jones and Phillips submitted). If rabbits are housed in a cage with a living area and darkened resting area, putting mirrors in the living area increases the time rabbits spend there, and especially the time that they spend investigating their environment and feeding (Jones and Phillips submitted).



Figure 10-3. A rabbit cage with a shelf, hay rack, food hopper and water bottle. It is possible to link two or more cages together and to move the shelf from one side to the other (photograph by Lena Lidfors 1996).

4.2 Housing in groups in floor pens

Rabbits can either be group housed in floor pens or in cages. In the latter case the limited area of a cage usually restrict this to pair-housing (Bigler and Oester 1994, Huls et al. 1991, Stauffacher 1993). This combines the benefits of cage housing, e.g. hygiene, experimental purposes, with animal welfare interest, and has been established in Switzerland, Germany, United Kingdom and Canada (Stauffacher et al. 1994).

Group housing of rabbits in floor pens has been introduced in many laboratories and countries during the last 10-15 years (Figure 10-4). This is beneficial for the rabbits because they can express social behaviours and exercise (Heath and Stott 1990, Batchelor 1991). Abnormal behaviours and physiological conditions caused by small barren environments are also reduced, but there is an increased risk of fighting between rabbits (Morton et al. 1993). The major factors that have to be considered when group housing rabbits are: compatibility of individual animals, size of pens, stocking density, husbandry practices and environmental enrichment (Morton et al. 1993).



Figure 10-4. Group housing in floor pens at the University of Guelph in Canada (photograph by Lena Lidfors 1996).

Rabbits which are not compatible will fight when placed together in a group, and the greatest problems occur when placing adult males together (Morton et al. 1993). There are strain differences in aggressiveness, i.e. Dutch rabbits are more aggressive than New Zealand White, whereas Lops are more docile (Morton et al. 1993). Some of the small strains of rabbits may show more aggression than the larger strains (Stauffacher et al. 1994). Individual animals may be highly aggressive, and fights can occur for unknown reasons, even in groups that have been stable for a long time (Morton et al. 1993). Therefore, groups of rabbits need to be carefully selected and regularly monitored. The best option is to keep litter mates which have been kept together from weaning (Zain 1988). Groups of intact, mature females not intended for breeding can be kept together (Morton et al. 1993). In periods of rest, does, bucks, and older young kept in mixed groups congregate and snuggle against each other or engage in mutual grooming (Stauffacher 1992). Does have showed a weak preference for a large, enriched, solitary pen over a group pen, but a strong preference for a group pen over a smaller, barren, solitary pen (Held et al. 1995). Fighting can still occur in groups of does, and a dominant female in oestrus can mount and damage the skin on the backs of other females and harass the group (Morton et al. 1993). The degree of compatibility of grouped rabbits will depend on factors such as strain, individual characteristics, sex, age and weight, size and structuring of pens, methods of husbandry and the interest and ability of the animal technicians (Bell and Bray 1984, Zain 1988, Morton et al. 1993,

Stauffacher 1993). Aggression may be seen in group pens even after the establishment of the dominance hierarchy.

From around 10 weeks of age it may be necessary to house males individually to avoid fighting (Morton et al. 1993). Groups of males kept in proximity of females tend to fight and urinate more frequently (Portsmouth 1987). Castration of males kept for longer periods in the laboratory may be one solution to be able to keep them in groups. The practical experience from castration of males is that aggression is reduced and stable for a long time afterwards (Gunn pers. com. in Morton et al. 1993, Lindberg pers. com. 2003). Castration should be carried out when the males reach sexual maturity at 50-80 days of age depending on strain and food composition (Stauffacher et al. 1994). It should be carried out only by well-trained persons and always before the males start to show aggressive behaviour, 3-4 weeks after weaning at the latest (Stauffacher et al. 1994, Morton et al. 1993). The testicles move down during sexual maturation, but then are withdrawn again. However, one has to consider what type of research the animals will be used for, as castration has an effect on the animal's physiology.

An alternative if castration is not advised is to place individually caged male rabbits in an exercise arena at regular intervals (Figure 10-5). This allows them to move around on a larger floor surface, to investigate enrichment objects and to get the smell from other males that have been exercised before them. This has not been evaluated in the laboratory units, maybe because it will take time for the animal keepers to take out and return the rabbits according to a routine schedule. However, in the case where castration is not possible it may introduce an important enrichment for the rabbits.

Rabbits placed in group housing should be of the same sex, of similar size and if possible, related and grouped when young, i.e. around the time of weaning (Morton et al. 1993). When establishing new groups of rabbits in floor pens the best option is to wean and mix at the same time around 6 weeks of age and to place 6-10 rabbits, preferably of the same sex, in one group (Morton et al. 1993). The ideal situation is when animals are kept in stable groups from birth (Zain 1988, Stauffacher 1993). Small groups may be most stable (Love and Hammond 1991). Some animals do not appear to settle well in groups, either because they are too dominant and bully the others or are too timid and prone to be bullied (Morton et al. 1993). When putting together rabbits that have been caged for six months or more it may be difficult to avoid fighting or self-inflicted injuries (Morton et al. 1993). However, individually-caged adult female rabbits of peaceful strains can be paired successfully, preferably in structured cages (Stauffacher 1993,

Morton pers. com. in Stauffacher et al. 1994). It is very important to provide refuge and hiding places for subordinate animals (Morton et al. 1993).



Figure 10-5. A male Russian rabbit placed in an exercise arena on the floor in a laboratory in Sweden (photograph by Lena Lidfors 1995).

The number of rabbits kept in each group pen should not exceed 6-8 mature animals (Morton et al. 1993, Stauffacher et al. 1994). This is recommended so that the rabbits can be adequately monitored for signs of bullying and ill-health. Groups of up to 20 laboratory rabbits have been successfully managed as stock and for the production of polyclonal antibodies (Stauffacher et al. 1994). However, more research is needed on optimum and maximum group size (Stauffacher et al. 1994).

When keeping breeding rabbits in groups they should be composed of 4-6 females, one male and their offspring until they are weaned at 30 days of age (Stauffacher et al. 1994). The group breeding housing system and management has been developed in Switzerland (Stauffacher 1989) and used in agricultural rabbit farming, but to our knowledge not being used for breeding of laboratory rabbits.

The minimum area of a floor pen should be large enough for the rabbits of a particular weight and size to be able to carry out normal behaviour, especially locomotion. One problem with using weight and size as a criterion of determining floor area is that young animals move more and might need more space to carry out play behaviours (Stauffacher et al. 1994). In the Swiss legislation each rabbit must be able to hop some steps or to jump up and down onto a shelf. This may help the rabbits to maintain a level of fitness and reduce the occurrence of disuse osteoporosis (Morton et al. 1993). The working group on "Refinement in rabbit husbandry" recommend that rabbits kept in groups have a clear area of 20 000 cm² with an overall minimum floor area of 6 000 – 8 000 cm² per rabbit for groups up to 6 rabbits. If more than 6 rabbits are kept in the groups an extra space of 2 500 cm² per rabbit is recommended (Morton et al. 1993). The height of floor pens should be 1.25 m., as rabbits can jump very high, and enrichment objects should be placed so that they can not be used for jumping over walls.

If European wild rabbits are used for research they should be housed in floor pens with sand or straw substrate so that they can hop around and exercise (Bell 1999). The reason for this is that wild rabbits kept in small cages have developed weak backs probably due to a lack of exercise. Young wild rabbits, between 4 and 8 weeks of age, fare better when they are kept in groups (Bell 1999).

4.3 Feeding and water

Rabbits are crepuscular and in the wild they usually graze during their active periods at dawn and dusk (Lockley 1961), or during early morning and at night (Cheeke 1987). However, feeding laboratory rabbits in cages means that they are almost invariably not fed the diet of grass for which they evolved to utilize. However, there is little evidence that they prefer a grass diet to one based on compound feed (Leslie et al. 2004). Despite this, it is often beneficial to supplement their ration of proprietary compound pellet with dietary enrichment, which as well as providing adequate nutrients (NRC 1966), in particular fibre (Lehmann 1990), which will increase the time usefully spent in procuring their cage (Leslie et al. 2004). The visual stimulus of a varied diet is particularly important (Ruckebusch et al. 1971). A mixed diet is also a feature of natural herbivore feeding behaviour, due to their desire to sample regularly in case of the disappearance of one feed (Parsons et al 1994).

Several forms of dietary enrichments have been tried with a significant degree of success, including supplying fibrous food to reduce boredom: hay (Berthelsen and Hansen 1999), grass cubes or hay in a bottle (Lidfors 1997), and fresh grass (Leslie et al. 2004). The most useful enrichment is something to chew (Brummer 1975, Huls et al. 1991, Lidfors 1997, Berthelson and Hansen 1999) and high fibre objects are preferred, with hay or straw remaining an effective enrichment for long periods (Brummer 1975, Lidfors 1997). Hay and straw also cause less weight gain than proprietary fibre sticks or compressed grass cubes (Lidfors 1997). Abnormal maternal behaviours and trichophagia or fur-chewing (Brummer 1975, Mulder et al.

1992) are eliminated in caged rabbits when hay or straw is given. If the supplementary hay is ground, it is ineffective at reducing problem behaviours, demonstrating a need for long fibre (Mulder et al. 1992).

Water should always be available *ad libitum* to rabbits (Mader 1997). The need for water is 50-150 g per kg body weight, and rabbits raised on pellets need 50 g per kg body weight. More water is needed for growing animals and pregnant and lactating females. One rule of thumb is that the need for water per 24 h is 10 % of the body weight (Meredith 2000).

5. CARE AND HANDLING

It is important that all personnel that handle rabbits have learned how rabbits react to frightening sounds and handling in order to avoid injuries to both the rabbit and human handler. Rabbits that have had positive contact with humans and been handled with care previously will come to the front of the cage when opening the door and sit still when being lifted out. Similarly rabbits in floor housing will sit still when a person comes to pick them up. Rabbits that have been scared by humans or not been handled try to flee, and may injure themselves. The fear and distress may be communicated to other rabbits in the animal room (Beynen 1992).

Rabbits should be handled firm and gently, because if they sense insecurity they may struggle (Batchelor 1999). Lifting of rabbits of all ages should be by grasping the scruff of the neck in one hand while the other hand is placed under the rump to support the animal's weight (Batchelor 1999). Some authorities suggest that the rabbits ears should be grasped in addition to the scruff for greater control, but Batchelor (1999) have the experience that some rabbits react as if this was painful, which increase the likelihood of struggling in the rabbit. Rabbits should never be carried by the ears.

6. HEALTH AND DISEASE PROBLEMS

During the last thirty years the health situation of laboratory rabbits in general has moved from a position of disease problems that is still prevalent among many pet rabbits to a vastly improved level of health. The laboratory rabbits of today are bred, housed and cared for in such a manner that their overall health situation is much better than that of their ancestors a couple of decades ago.

The awareness among researchers and laboratory animal staff of the great importance of a good health situation for the animals has contributed to improvements in hygiene and in health monitoring systems, which in turn have led to a good general health situation among laboratory rabbits. The establishment of SPF breeding colonies in the late 1970s and early 1980s gave the rabbits an immensely improved health situation (Eveleigh et al. 1984). The first guidelines of FELASA (Federation of European Laboratory Animal Associations) for health monitoring of breeding colonies on a regular basis were also a milestone in the improvement of health for laboratory animals. At this time, in the mid-1980s, health discussions mainly dealt with hygienic aspects of health such as infectious diseases of different sorts and how the animals and the research results were affected by the infections.

6.1 General health

Clinically healthy rabbits have a well-groomed fur, alert and clear eyes, and quick reflexes of escape if threatened. When picked up and held by the caretaker a healthy rabbit accustomed to handling by human beings will give the impression of strong muscles in a resting state. When checking the health of laboratory rabbits on a daily basis the major points are the posture of the animal, look of eyes, ears and nose for signs of discharge and other abnormalities, the state of the fur (especially in group-housed rabbits since the effects of fighting might only show as a minor flaw in the fur), and the look of faeces and urine. Normal faeces should consist of dry fecal pellets of a uniform size and normal urine can vary in colour from yellow to dark red and is often cloudy due to excretion of calcium in the urine. See Meredith (1998) for more information.

FELASA has issued guidelines (Nicklas et al. 2002) on how to monitor the health status of laboratory rabbits and rodents. These guidelines comprise infectious agents, frequency of sampling, sample sizes and preferred methods of analysis for complete monitoring of the hygienic state of a breeding colony. The result of following the FELASA guidelines for health monitoring of laboratory rabbits and rodents can be called a Health Defined Rabbit. There are similar terms of earlier origin that are sometimes used for the same purpose, i.e. SPF (Specific Pathogen Free) or VAF (Virus Antibody Free). These different terms aim to give us information of the quality of health of the rabbits microbiologically.

A special health problem has been introduced along with the use of certain strains of rabbits with a genetically transmitted disease trait such as the WHHL (Watanabe Heritable Hyperlipidemic rabbit). This rabbit is characterized by the development of atherosclerotic lesions in arteries. Older rabbits tend to accumulate calcium deposits in various parts of the body, which of course affects their well-being.

Many diseases will not show clinical symptoms unless the rabbits are compromised by a number of different stressors at the same time, where all factors influencing the animals well-being positively and negatively add up (Nerem 1980).

6.2 Infectious diseases

Bacterial, viral or parasitic agents cause infectious diseases that rabbits can be affected by. Many of the infections of laboratory rabbits are subclinical, especially viral infections and may pass unnoticed, mainly affecting the results of studies performed. In the literature, there are plenty of data about diseases of the rabbits, the literature used here include: Harkness and Wagner 1995, Laber-Laird et al. 1996, and Hillyer and Quesenberry 1997.

Bacterial agents including *Pasteurella multocida* and *Bordetella bronchiseptica* are the main cause of respiratory inflammations, which may give symptoms such as sneezing, coughing, nasal discharge and lethargy. *Pasteurella multocida* and *Staphylococcus aureus* may also be involved in formation of abscesses in subcutaneous tissues, behind the eye bulb or in internal organs as well as inflammation of the mucus membranes of the eyes and in the middle ear. Other bacterial infections of rabbits are eye infections by *Moraxella catarrhalis*.

Infections of the gut by bacteria include mucoid enteritis that affects mainly young rabbits. The symptoms are depression, anorexia, diarrhoea and mucus in the stool and the cause is multifactorial with the bacterium *Clostridium spiroforme* being one of the major factors. *Escherichia coli* and other strains of *Clostridia* may also cause enteritis with diarrhoea as main symptom. Inflammations of the gut are aggravated by nutritional imbalance with a deficit of dietary fibres.

Viral infections comprise mainly viruses that affect the digestive tract. Rotavirus and rabbit enteric coronavirus may give the rabbits mild diarrhoea. Rabbit Viral Hemorrhagic Disease (RVHD) is a feared disease among pet rabbits but it is unlikely to infect laboratory rabbits. This disease affects many organs and the main symptoms are lethargy, anorexia, diarrhoea and haemorrhage from body openings such as the nose and urogenital opening.

Myxoma virus could be transferred to laboratory rabbits but this requires the presence of a vector, most often fleas but other insects may also act as vectors for myxoma virus. Myxomatosis is common in wild rabbits and could possibly infect laboratory rabbits in areas where wild rabbits are common.

Parasites that infect laboratory rabbits are mainly endoparasites. The largest problems are created by coccidiosis. This disease is caused by different strains of *Eimeria*. One strain, *E. stiedae*, infects the liver and

causes different degrees of symptoms ranging from unapparent retardation of growth to fatal disease. Other *Eimeria* strains such as *E. perforans* and *E. magna* infect the intestine of rabbits. Symptoms depend on the amount of coccidia present in the gut and of the susceptibility of the rabbit. Most often, only the youngest animals show symptoms whereas in older rabbits coccidiosis is sub-clinical. The symptoms include weight loss and mild intermittent to severe diarrhoea.

Pinworms, *Passalurus ambiguus*, colonize the caecum and colon and the eggs are passed in the faeces. Pinworm infections are generally without symptoms.

Encephalitozoonosis or nosematosis is a disease common in pet rabbits and wild rabbit and regularly occurs in laboratory rabbits. It is caused by *Encephalitozoon cuniculi*, an intracellular protozoan that is transmitted from the urine of infected animals via the oral route to the intestine and tissues of susceptible rabbits. The parasite mainly damages the kidneys and the brain of infected animals but most often no symptoms are seen clinically.

Ectoparasites are uncommon in laboratory rabbits but a few should be mentioned since they are common in other rabbits. Ear mites, *Psoroptes cuniculi*, can cause considerable itching and wounds on and around the ears. *Cheyletiella parasitivorax* is the fur mite of rabbits. Mites, fleas and lice of rabbits cause considerable suffering since they produce anaemia and/or pruritus and result in generally poor condition in the rabbit.

6.3 Traumatic injuries

Fighting is the most common cause of traumatic injuries. Fighting can occur between all sexually mature males, between females that are not acquainted to another, between individuals in overcrowded pens if the feed hoppers and water bottles are not in sufficient numbers. Fighting males are very aggressive and may cause considerable damage to each other. Wounds may show readily but may also pass unnoticed, concealed by fur.

Fractures are uncommon and may result from handling cage-housed rabbits unaccustomed to being handled which cause them to struggle forcefully, and it mainly occurs in cage-housed rabbits with a weak skeletal structure in the vertebral column (Rothfritz 1992).

6.4 Diseases caused by housing, feeding and breeding

Housing and hygienic routines have a strong impact on the health of laboratory rabbits. Solid-bottom cages and pens need thorough cleaning and regular disinfections so as not to spread intestinal parasites and bacteria back to the rabbits. Cage-floors with perforation for droppings onto a pan reduce the number of coccidian spores etc. that the animals can ingest. "Sore hocks" is a condition seen in heavier breeds of rabbits kept on solid-bottom cages or pens with inappropriate hygiene and the symptoms are bleeding and infected wounds along the hind feet of the animals.

The diet of laboratory rabbits is crucial for the microbial balance in the gut and intestinal morphology (Yu and Chiou 1996) and thus the optimal rate of dietary fibres are essential to keep the animals from developing soft stool or diarrhoea.

Another type of health problem arises from the use of certain types of diets aiming to inflict metabolic changes in the animals, for example high cholesterol diets for the development of atherosclerosis. A side effect of using these diets in long-term studies is fatty liver and deposits of cholesterol in various organs.

7. EXPERIMENTAL TECHNIQUES

Rabbits are often used for collecting blood in immunisation studies. Blood can be taken from the marginal ear vein or by cardiac puncture. In the former case dilation of the ear vein facilitates the removal of blood, and with good blood flow up to 10 ml blood may be obtained (Batchelor 1999). Local anaesthetic facilitates the removal of blood from those animals which are distressed by the insertion of the needle into the ear vein. The cardiac puncture must be carried out under anaesthesia with the animal in dorsal recumbency. Rabbits have about 70 ml of blood per kg body weight, and a maximum of 7 ml/kg body weight should be taken at a single sample (Batchelor 1999). Others have suggested that up to 8 ml of blood could be taken per kg body weight at a single sample (Iwarsson et al. 1994)

The body temperature of rabbits should be taken by a suitable thermometer which has been lubricated for ease of introduction into the anus (Batchelor 1999). The rabbit should be gently restrained, the tail lifted and the thermometer gently inserted. The thermometer should never be forced into the anus, and if there are any obstructions to the passage it should be investigated. The thermometer should be left in situ for 1-2 minutes, whereafter the temperature is recorded. In rabbits the normal mean rectal temperature is 38.3°C with a range of 37.0-39.4 °C.

Rabbits may be given different substances by oral (p.o.), subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.) or intraperitoneal (i.p.) administration. For more detailed description of these techniques see Batchelor 1999 and Iwarsson et al. 1994.

For anaesthesia of rabbits there are many different methods and substances (Svendsen 1994). Depending on the purpose of the procedure or

operation a specific substance should be chosen, and then the recommended range of doses given in the literature should be followed (Olfert et al. 1993, Svendsen 1994, Batchelor 1999). Generally relief of pain should be given to all rabbits during operational procedures, if there is evidence that pain is present in the individual.

Postoperative care of rabbits includes placing them in a recovery cage, the box they were placed in before surgery or the home cage which has been lined with a tray liner (Batchelor 1999). The liner should be folded over the animal and a cotton surgical drape placed over it to minimise hypothermia. A 'Vet Bed', which is commercially available may also be used. The liner or 'Vet Bed' should be removed about 30 minutes after the animal has regained consciousness and is sitting up. When operating on several group housed animals the last animal operated on must have completely recovered consciousness before all the animals are returned to the pen simultaneously (Batchelor 1999). If animals are returned to a group pen while they recover consciousness they may be subjected to aggression. Incision sites should be covered with a clear plastic dressing spray, but rabbits may occasionally interfere with their stitches. A plastic collar can then be applied to the animal to restrict its access to the operation site after suturing (Batchelor 1999).

Euthanasia of rabbits is usually carried out by intravenous injection of an overdose of barbiturates, such as sodium pentobarbitone (Batchelor 1999). Physical dislocation of the neck is another option for rabbits up to one kg in body weight. However, it is recommended that this always is followed by heart puncture or cutting one of the main vessels so that the animal is drained of blood.

8. CONCLUSIONS

In order to ensure the welfare of laboratory rabbits there are several aspects to consider. First of all one has to make sure that the rabbits are purchased from a breeder producing health defined rabbits with a controlled genetic background, and with an enriched housing. The transport to the laboratory should be as short and stress-free as possible. Placing two rabbits in the transport box could reduce stress. At arrival to the laboratory the rabbits should be checked for any health problems and placed in its new cage. Acclimatization should be one to two weeks. Young rabbits could be housed pair-wise in cages, or in larger groups in floor pens. Cages should be large enough to give rabbits a place to perform hopping movements and to lie in a position fully stretched out. They should be provided with free access to hay and water, and fed at least once daily. Floor pens should be large enough to house the maximum number of rabbits planned for, at least one

meter high to prevent escape and enriched with refuges, hay, gnawing sticks, etc. Regular cleaning of cages and floor pens are important to keep a good hygiene and healthy animals.

There has been relatively large amount of research on the welfare of laboratory rabbits over the past 20 years (Morton et al. 1993, Gunn 1994, Stauffacher et al. 1994, Hubrecht et al. 1999). Both improvements of cage systems and floor housing have been evaluated. There are several companies selling environmentally enriched cage systems and equipment for building floor pens and enrichment items for floor pens. Parallel with this development, new regulations have been planned and intensively discussed over the past years (Appendix A of the European Convention, regulations in Sweden), and some have been put into practice (regulations in Switzerland and UK). The most important welfare research to carry out for the future would be to verify if some of the behaviours rabbits perform in semi-natural environments, for example digging, is so important that it could be considered an essential behavioural need. Alternatives to permanent cage housing of mature bucks, as for example regular exercise in an enriched area, should also be scientifically evaluated. There is also a need for more controlled experiments on the effects of group housing and environmental enrichment on the results of laboratory research.

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