

### Specialist information

## From the Committee for Laboratory Animal Nutrition

# Feeding concepts and methods in the laboratory animal facility and in animal experiments

- Rabbits -

Rabbit (*Oryctolagus cuniculus* L.) – at the example of the breed New Zealand White

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#### **Table of contents**

Preliminary remarks	3
Life phases	3
Growth	3
Oestrus and pregnancy	5
Lactation	5
Housing	5
Supplementary feed	7
Feeding technique	7
Peculiarities of digestion	8
Stomach	8
Caecum	8
Caecotrophy	8
Intestinal microflora	10
Feeding in experiments	10
Water supply	10
Diseases	11
Enrichment	11
Transport	12
References	13

#### Keywords:

Rabbits – Life phases - Feeding – Peculiarities of digestion - Diseases – Enrichment

#### **Preliminary remarks**

The numerous rabbit breeds today are descended from the European wild rabbit (*Oryctolagus cuniculus* L.). Rabbits are lagomorphs (belonging to the order *Lagomorpha*). In the upper and lower jaw, they have two large incisors (cutting teeth) each, behind which in the upper jaw there is another pair of small peg incisors. All rabbit teeth are open-rooted. This means the roots are not coated with cement (Wolf and Kamphues 1996). The teeth thus grow throughout life, growth being slightly faster in the lower jaw than in the upper jaw (about 1.1-1.8 mm per week in the upper jaw and 1.3-1.7 mm per week in the lower jaw). The incisors have an enamel coating on the rostral surface, which accounts for their chisel shape and sharpness (Zinke 2004).

#### Life phases

#### Growth

Rabbits are born with a live mass (LM) of 40 - 60 g (Table 1). Data from birth to week 5 and from the week 14 are only available as individual values and are therefore not listed (Fig. 1). During first two weeks after birth, the young feed exclusively on the mother's milk. The daily intake of milk initially amounts to up to 30% of live mass (Schlolaut 2003). The amount of milk produced by the mother is clearly correlated with the size of the litter and can vary considerably. Young rabbits are suckled by the mother only once, or in rare cases twice, daily (Hoy 2006) for three to five minutes (Table 2). This is possible, because rabbit milk has a very high energy content (9.0 MJ/kg; by comparison, the value for cow's milk is 3.17 MJ/kg) (Schlolaut 2003; Manning 1994).

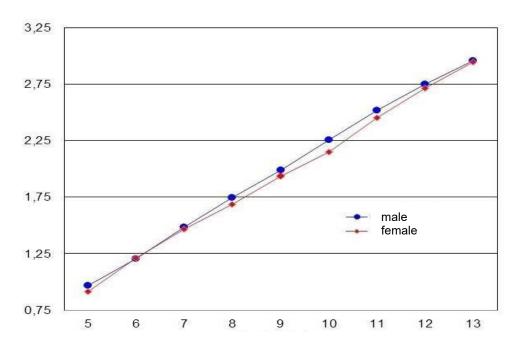


Figure 1: Development of average live mass of the rabbit (Crl: KBL (NZW); Charles River Laboratories 2012).

Table 1: Data relevant to feeding in rabbits (*Oryctolagus cuniculus L.*), modified according to: Baumgartner (1999), Charles River Laboratories (1999), Schall (2008), Winkelmann (2006), Zumbrock (2002).

Live mass at birth	40 - 60 g
Live mass when weaned	550 - 800 g
Sexual maturity	20 weeks
Breeding maturity	16 weeks
Gestation	30 - 32 days
Life expectancy	8 -12 years
Litter size	4 - 10
Oestrus cycle	no regular cycle, post-partum oestrus (follicles mature continuously; induced ovulation, breeding possible all year)
Live mass	2 - 6 kg
Loss of live mass during transport	up to 5 hours: ~10%, up to 10 hours: ~15%
Food intake	50 - 100 g dry matter / kg LM / day
Water intake	50 - 100 mL / kg LM / day or more (pregnancy)

Table 2: Average food intake of juvenile and adult rabbits (*Oryctolagus cuniculus L.*), modified according to: Baumgartner (1999), Zumbrock (2002), Schlolaut (2003), GV-SOLAS Committee for Humane Laboratory Animal Housing (2010).

Age (weeks)	Live mass (LM) (g)	Food intake/day (g/kg LM)	Meals/day (n)
Birth	40 – 60	1 - 2 ml **	1 - 2
4	500 - 600		
6	1000 - 1200	70 - 90*	40 - 50
8	1600 - 1800	60 - 70 *	
10	2200 - 2400	50 - 60 *	
13	2900 - 3100	45 - 55 *	25 - 30
Adult	3800 - 5000	30 - 50 *	

<sup>\*</sup> based on pelleted complete feed with ≤ 10% residual moisture; \*\* based on mother's milk

The ability to form lipase and amylase is still underdeveloped at 21 days, reaching only 12% of the values found in animals aged 35 days. This means that fat and starch in the feed are insufficiently digested. Some authors interpret this as a possible cause of bacterial enteritis (dysentery), which occurs relatively often in rabbits that are weaned early.

#### **Oestrus and pregnancy**

Rabbits do not have a marked sexual cycle; there is no spontaneous egg maturation and ovulation. A female rabbit usually shows post-partum oestrus a few hours after giving birth to her litter. Ovulation itself is induced by the mating act (provoked ovulation) and occurs about 10 hours after the act (Homeier 2005). Ovarian follicles (Graafian follicles) continuously grow and regress.

#### Lactation

With a maximum lactation period of 35 days, depending on weaning age, an average of 250 to 300 g milk is produced daily. Peak lactation is between the 18th and 23rd day. On average, the milk contains 30.7% dry matter, 12.7% raw protein, 14.8% raw fat, 0.9% lactose and 2.3% raw ash (Schlolaut 2003; Kamphues et al. 2009).

During the suckling period, between days 12 and 14 after birth, the young begin slowly to feed independently. The mother's high consumption of nutrients as a result of lactation must be made up by an increase in food intake (Table 3) and higher nutrient contents of the feed (Table 4).

The fat content of the ration for the lactating female is particularly important: a higher proportion of fat ensures a better energy supply, while at the same time exerting a positive influence on the taste of the feed and hence on food intake. This allows the increased protein and energy needs to be taken into account without having to reduce the fibre content of the feed (Table 4). Otherwise, it would lead to digestive disorders. Of the fatty acids, linoleic acid is essential and should make up 0.5% of the dry feed.

The comparatively high fat, protein and mineral content takes into account the lack of both the thermal insulation with only once-daily suckling and the rapid growth during the first weeks of life.

Table 3: Average food and water intake of pregnant and lactating rabbits (*Oryctolagus cuniculus L.*); Schlolaut (2003), Weiss et al. (2014).

Pregnancy (days)	Live mass (LM) (g)	Food intake/day (g/kg LM)	Water intake/day (ml/kg LM)
1 – 21	4200	50	120 - 250
22 – birth	4500	70 - 80	120 - 250
Lactation	4200	120	250 - 500

#### Housing

This phase extends from the end of the growth phase to the end of life without any special requirements. Food intake and composition during the housing phase are shown in Tables 1, 2 and 4.

Table 4: Nutrient contents of commercially available complete feed for rabbits (*Oryctolagus cuniculus L.*) in percent<sup>1</sup>

		Feed Breeding	Feeding Housing
Energy			
Digestible energy (DE)	MJ/kg	13.0	12.6
Raw nutrients			
Dry matter	%	89.0 - 89.5	89.0 - 9.3
Raw protein	%	17- 22.5	13.5 - 7.5
Raw fat	%	3.0 - 6.0	2.5 - 4.0
Raw fibre	%	14.5 - 15.5	14.5 - 4.5
Raw ash	%	5.8 - 8.0	7.2 - 8.5
Minerals			
Calcium	%	0.7 - 1.1	0.7 - 1.0
Phosphorus	%	0.4 - 0.7	0.4 - 0.7
Sodium	%	0.19 - 0.30	0.20 - 0.31
Magnesium	%	0.20 - 0.32	0.20 - 0.32
Potassium	%	1.20 - 1.50	1.10 - 1.72

<sup>&</sup>lt;sup>1</sup> As described in the text, no absolute recommended values are given for nutrients.

Feed with a crude protein content of more than 16% and a raw fibre content of less than 16% should be administered, by way of exception, only in the growth and lactation phase (Table 4).

The comparatively large stomach of the rabbit allows the comparatively low nutrient content caused by the high raw fibre content to be offset by higher food intake (Table 3). The currently standard nutrient contents of protein, fat, raw fibre, minerals and energy for complete feed are indicated in Table 4. These nutrient contents serve as a guideline and should be regarded as such.

If breeding feed is provided ad libitum during the housing phase, the animals may become fat and arteriosclerotic changes may occur, especially in the aorta and coronary arteries. Increased cases of dysentery and death must also be expected.

#### Supplementary feed

Feeding with untreated hay is hygienically problematic (introduction of wild rodents, adhesion of durable forms of parasites). Autoclaved hay is not accepted in most cases because of residual moisture and associated lack of bite. Acceptance is good, however, if the hay is subsequently aired. GV-SOLAS (Committee for Humane Laboratory Animal Housing 2010) recommends straw and hay as supplementary feed and also for activities, but only when heated to max. 80°C / 4 hours. If possible, the rabbits should preferably be provided with hay sanitized by means of ionizing radiation.

One way of including this with the feed that has proved successful is to add it to feeding racks in the form of straw and hay pellets (cobs or briquettes). Pellets are seen less as supplementary feed but serve primarily as a means of activity for the animals (enrichment).

#### Feeding technique

Rabbits are usually offered food ad libitum, because they take 25 - 80 small meals spread over the course of the day (Schwabe 1995) with increased intake between 17.00 and 23.00 h (Zumbrock 2002).

Since singly housed animals frequently eat more than they need, they would become fat if they were housed in this way for a prolonged period and with ad libitum feeding. They should therefore be fed restrictively (Van Zutphen et al. 2003; GV-SOLAS Committee for Humane Laboratory Animal Housing 2010; Weiss et al. 2014).

There are two possibilities for administering food:

- The food is supplied from the cages through a feed container attached to the outside
  of the cage. This has the advantage that food intake can be easily monitored. Further
  advantages of this approach are the opportunities for a short-tern change or
  deprivation of food if necessary, e.g. during an experiment.
  This approach is also better for cleaning the feed container.
- Automatic feeding systems are made of stainless steel and plastic and run centrally above the cages. A vertical pipe runs from the central strand into the cage and ends in a feeding bowl accessible from all sides. This allows four animals to feed unhindered by each other. The feed is automatically replenished when it runs low. The disadvantages are that food intake is not monitored and the hygiene and cleaning procedures are more laborious. In addition, the technical functions must be regularly checked.

In the case of group housing, attention must be paid to ensuring the number of feed containers provided is appropriate to the size of the group, so that all rabbits have free access to the feed. Experience shows that one container is sufficient for four animals (Sandór and Warncke 2014).

#### **Peculiarities of digestion**

The digestive tract of the rabbit (Fig. 2) shows a number of anatomical and physiological peculiarities that are to be seen as an adaptation to the purely plant-based, high-fibre diet that is mostly low in protein and water in nature.

#### Stomach

Rabbits possess a monogastric, thin-walled stomach. Compared with other herbivores, the stomach is relatively large, accounting for about 35% (100 - 125 cm³) of the total capacity of all the digestive organs. Since the stomach itself is only muscled to a limited extent, the stomach contents are emptied into the small intestine predominantly through the pressure resulting from the continuous delivery of food. There are no natural fasting phases.

Transit time in the stomach is shorter with a larger particle size of feed, so that the swallowing of fur when grooming does not lead to the formation of fur balls (trichobezoars; Schall 2008). Fur balls are a danger that can lead to death in rabbits.

#### Caecum

The caecum in rabbits is enlarged into a large fermentation chamber. Compared with other animal species, the caecum is remarkably large, accounting for 40-45% of the total capacity of the digestive organs, which is greater than the capacity of the stomach (Fig. 2). A separation mechanism also comes into play in the caecum. While finer particles are initially retained and later eliminated as caecotrophs (or caecal pellets), coarser particles are excreted immediately with the hard faeces (Hörnicke 1978).

The enzymatic digestion in the small intestine is complemented in the caecum by the microbial breakdown of the pre-digested contents. Plant components that are difficult to digest are broken down with the aid of bacterial cellulase. Aside from optimizing the utilization of energy (20-30% of the energy needs of a rabbit are met by bacteria alone), bacteria are also responsible for the formation of B vitamins and vitamin K.

#### Caecotrophy

A peculiarity of the rabbit is caecotrophy. This refers to the regular intake of a particular form of faeces (caecal or soft faeces) by some species of herbivores, which results in a more efficient utilization of plant food that is difficult to digest (Schlolaut 2003; Hirakawa 2001; Sharp, 2007).

Rabbits produce two forms of faeces in a circadian rhythm:

- During the day, they excrete normal, dark faeces in the form of small, dry balls (hard faeces), which does not contain any usable nutritional residue and is not eaten.
- During the resting period, however, rabbits excrete so-called soft faeces: soft, wet and mostly lighter balls covered with a layer of mucin, which are known as caecotrophs. The animals consume this again, taking it up in the mouth directly from the anus.

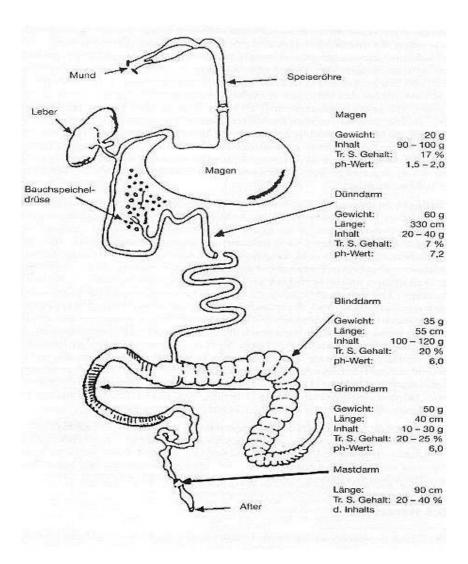


Figure 2\*: The digestive organs of the rabbit *(Oryctolagus cuniculus L.);* Fekete (1993). The numerical values refer to a 12-week-old animal fed on complete feed. Illustration by kind permission of DLG-Verlags GmbH

The caecotrophs that are eaten are transported via the anterior fundus section of the stomach to the caecum, where they undergo further bacterial fermentation over the course of several hours. The soft faeces consist of undigested food, vitamins, microbial protein and water. Calculation of the water contained in the faeces excreted during the day as a proportion of the total water loss yields values of ~2%, whereas the water loss of the caecotrophs excreted during the night amounts to 6-8% (Tschudin 2010; Warncke 2012). Overall, caecotrophs account for about 25-30% of all the faeces.

The protein produced by caecal bacteria contributes substantially to the protein supply of the rabbit. The raw fibre content of the caecotrophs varies between 10 and 15% of dry matter (Zumbrock 2002).

#### Intestinal microflora

The intestinal flora provide for the physiological digestive functions (Manning 1994; Matthes 1969). In the healthy rabbit, these flora consist mainly of Gram-positive bacilli, Gram-positive, anaerobic lactobacilli and to a lesser extent Gram-negative, anaerobic species of Bacteroides.

The healthy balance - or eubiosis - of the intestinal microflora not only provides for normal digestive functions, but also serves as a barrier against colonization by pathogenic microorganisms.

#### Feeding in experiments

Since rabbits are not capable of vomiting or regurgitation, it is not absolutely essential to fast the animals before anaesthesia/operation without further experiment-related reasons (GV-SOLAS Committee for Anaesthesia 2012). Experience shows that, in rabbits deprived of food for a prolonged period, there is a greater tendency for hypoglycaemia to develop during the operation and dysbiosis with gastrointestinal disorders to occur postoperatively (Henke 2012). The growth of pathogenic bacteria leads to the development of enterotoxaemia.

Prolonged fasting has a serious effect on the digestive processes and causes considerable stress to the animals (GV-SOLAS Committee for Anaesthesia 2012). Recovery is also noticeably slower in rabbits that did not receive food before the operation.

Rabbits have a tendency to retain food and water in the oropharyngeal space. Experience has shown that removing access to food about one hour before anaesthesia ensures that the pharyngeal space no longer contains any residues of food and the stomach is not overfull. A rabbit should be provided with food and water again immediately after waking from anaesthesia. By ensuring that conditions in the experiment are adapted in good time to conditions in the housing facility, the frequency of disease can be markedly reduced.

When certain analgesics are administered, e.g. Buprenorphin® (from the group of opioids), it must be borne in mind that these can cause postoperative changes in behaviour, including restricted movement (Henke, 2011), which can also lead to disturbances of food intake.

#### Water supply

The rabbit has a high water requirement in relation to its body mass (Lang, 1981), the amount of water required depending on the intake of dry matter and raw fibre. Coenen (1999) and Winkelmann (2006) put the water requirement at 10 mL / 100 g LM / day. Any restriction of water intake results in reduced food intake and can lead to an accumulation of the colon content as a result of the water restriction. A further risk of insufficient water intake lies in the danger of urinary stones forming in the case of a high calcium intake (Kamphues 1999; Wolf and Kamphues 1995).

#### **Diseases**

Feeding errors (perished, contaminated or incorrectly composed feed), diseases, orally administered antibiotics and unfavourable housing conditions (inadequate hygiene of cage, feeding and drinking water systems, high humidity or wet conditions, elevated ambient temperatures and strong airflow) can affect the homeostasis of the digestive system and lead to dysbiosis as a result of colonization by potential pathogens (e.g. cocci, *Clostridium piliforme*, *E. coli* and yeasts; Matthes 2002).

Lack of adequate amounts of raw fibre not only induces digestive disorders but can also lead to excessively long incisors or bridge formation in the cheek teeth due to inadequate abrasiveness of the continuously growing teeth (Wolf 2009).

Tympanites after the ingestion of rotting or fermenting feed, as well as abrupt changes of feed (Weiss et al. 2014), and acute gastric overload resulting from the voracious intake of large quantities of food are common disorders in rabbits (Sharp, 2007). In most cases, they indicate respiratory depression and circulatory failure, which often lead to the death of the animal. Rupture of the stomach has also been observed in a few cases.

At the first signs of dysentery (softer consistency of the faeces and marked, characteristic odour in the animal room), it is often useful to withdraw the pelleted maintenance feed and give an energy-reduced, high-fibre maintenance feed for 7–10 days or simply good-quality hay or hay briquettes for up to 3 days. After this, the usual feed should be gradually restored.

Using of feedstuffs from sterile production and acidifying the drinking water to pH 2-2.5 help to reduce cases of dysentery and losses. The teeth should be checked for a possible influence on the enamel. Effective support can also be provided in the form of regular and comprehensive hygiene measures in the area of food and water containers or systems, especially if these are automated.

Unlike with other animal species, calcium in the feed is completely absorbed. If the intake is higher than the requirement, it is excreted again in the urine, but without forcing renal elimination of water (Kamphues 1999). A calcium surplus is clearly identifiable by the viscous, gritty consistency of the urine. To minimize the risk of urolithiasis (primarily calcium-rich stones in this case) or calcification of the vessels, the administration of calcium should therefore be based strictly on the animal's needs (Table 4).

The eating of foreign bodies (inappropriate enrichment) and hairs leads to chronic gastric overload in some cases.

#### **Enrichment**

Both hay and straw - irradiated as far as possible - offer suitable opportunities for activity or, as an alternative, pieces of wood in various shapes and sizes for gnawing (GV-SOLAS Committee for Humane Laboratory Animal Husbandry 2010); these should always consist of a soft wood, however, such as poplar or aspen (GV-SOLAS Committee for Humane Laboratory Animal Husbandry 2010; Schwabe 1995). Wood for gnawing can help prevent gingival bleeding and inflammation (Princz et al. 2008). It is advised against using steel rattles, because

these can often lead to dental fractures and injuries to the mouth/lip region of the animals. The result in most cases is a reduction of food intake.

#### **Transport**

The loss of live mass resulting from transportation can amount to 5-15 percent. This is heavily dependent on the duration and conditions of transport (ambient temperature, relative humidity, capacity and stocking density of the transport container) during the journey (Charles River Laboratories 1999; Warncke 2012). According to the Animal Welfare Transport Ordinance (TierSchTrV 2009), the dispensing of food and drinking water is not necessary for transport times of up to 12 hours.

Losses and intestinal diseases after longer transport times are often due to a change of food and of the drinking installations during and after the journey, as well as to the short period of familiarization with the hygiene conditions to the new housing facilities.

In older animals, no problems are to be expected with the change of food as a rule. In the case of younger animals or when continuity is needed because of study requirements, use of the same feed as in the original housing facility or the gradual withdrawal of old feed until the changeover is complete should be considered.

Alternatively, both feed regimens may be provided in parallel over a defined period, so that there is a gradual and natural adaptation to the new feed. This has the additional advantage that the time-consuming mixing of the feed is avoided (Warncke 2014).

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