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Characterization and manufacturing processes for laboratory animal nutrition

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Contents

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1. Foreword

This document is intended to give people who work in laboratory animal science an overview of the different types of laboratory animal feed and the manufacturing processes used for these feeds. In addition to the definition of common terms from the applicable provisions of feed law and the characterization of different feed types, individual aspects of production are also addressed. The latter is intended mainly to provide information on possible nutritional changes in the ingredients and also possible negative impacts of test substances resulting from the manufacturing process.

Unexpected reactions in animals during an experiment are often associated with deficient production of the feed. But this is only one of many possible causes. If problems occur or there are any uncertainties, therefore, the feed producer always be contacted as soon as possible for consultation. A closer understanding of the production technology can also help to understand the significance of the feed for the experimental procedure.

2. Definitions according to feed law

The provisions of feed law applicable in Germany and the EU serve as the basis for the characterization of laboratory animal feed and the processes used for manufacturing this feed. These regulations cover, among other aspects:

- the ingredients and additives of feed
- the production of feed
- the characterization of feed
- the placing of feed on the market
- the guidelines for official sampling and analysis of feeds

Note: The definitions listed below are taken from the above-mentioned legal texts in their current version (as of July 2019). This information is not subject to any updating service on the part of GV-SOLAS.

2.1. Feed

(Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety)

Feed is defined throughout the EU as “any substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals.” A distinction is drawn between feed materials and compound feed.

2.2. Feed materials

(Regulation (EC) No 767/2009 on the placing on the market and use of feed)

Feed materials are “products of vegetable or animal origin, whose principal purpose is to meet animals’ nutritional needs, in their natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, whether or not containing feed additives, which are intended for use in oral animal-feeding either directly as such, or after processing, or in the preparation of compound feed, or as carrier of premixtures". These include, for example, forage such as hay, straw or silage, cereal grains and products, fruits, vegetables, vegetable oils and fats, minerals, but also milk products or fish meal. A positive list of the feed materials approved in Europe can be found in Part C of Regulation (EU) No 68/2013 on the Catalogue of feed materials. Feed materials can be fed directly or processed as components in compound feed.

2.3. Compound feed

(Regulation (EC) No 767/2009 on the placing on the market and use of feed)

Compound feed is "a mixture of at least two feed materials, whether or not containing feed additives, for oral animal-feeding in the form of complete or complementary feed." For example, a compound feed for mice and rats that is classically used in laboratory animal facilities usually consists of several feed materials (various cereals, soya, minerals) and additives (trace elements, vitamins).

2.4. Complete feed

(Regulation (EC) No 767/2009 on the placing on the market and use of feed)

Complete feed is a "compound feed which, by reason of its composition, is sufficient for a daily ration." Complete feeds are thus intended to fully meet the animal's daily nutritional requirements without any need to supplement the ration with other feeds. Most commercially available laboratory animal feeds are complete feeds.

2.5. Complementary feed

(Regulation (EC) No 767/2009 on the placing on the market and use of feed)

Complementary feed is a "compound feed which has a high content of certain substances but which, by reason of its composition, is sufficient for a daily ration only if used in combination with other feed." Complementary feeds are used in combination with other feeds to meet the animal's nutritional requirements (e.g. mineral feeds).

2.6. Ingredients

(German Feed Regulations)

Ingredients are "Substances - other than feed additives, residues (pesticides - author's note) and undesirable substances - which are contained in a feed material or compound feed, and which influence its feed value (...)". These include the nutrient fractions typically included in feed analysis (crude protein, crude fat, crude fibre and crude ash) as well as other value-determining nutrients, such as calcium, phosphorus, vitamin A, vitamin B12, etc.

2.7. Feed additives

(Regulation (EC) No 1831/2003 on additives for use in animal nutrition)

Feed additives are “substances, micro-organisms, or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the functions” listed in Article 5 of Regulation (EC) No 1831/2003: e.g. improvement in the characteristics of the feed, fulfilment of nutritional requirements or improvement in digestibility. A feed additive must be approved for the target species concerned. A daily updated list (positive list) of approved feed additives is available from the responsible German federal department. They include vitamins, trace elements, amino acids, binders, anti-caking agents, preservatives, flavourings or colorants, which are added to the feed for a specific purpose and in a specified quantity.

2.8. Medicated feed

(§56 German Medicinal Products Act)

Medicated feeds “are medicinal products in ready-to-feed form, which are manufactured from medicated premixes and compound feeds, and which are intended to be placed on the market for use in animals”. Accordingly, from a purely legal point of view, it is no longer a feed, but a medicinal product. Pursuant to § 56 of the Medicinal Products Act (AMG), the medicated premix mixed into the feed must be approved for this purpose. The decisive factor for a medicated feed is the therapeutic indication (e.g. fenbendazole medicated feed for herd therapy in case of pinworm infestation). However, feed to which a test substance is added for experimental purposes is not a medicated feed.

2.9. Undesirable substances

(Directive 2002/32/EC on undesirable substances in animal feed)

Undesirable substances are “any substance or product, with the exception of pathogenic agents, which is present in and/or on the product intended for animal feed and which presents a potential danger to animal or human health or to the environment or could adversely affect livestock production.” These include, for example, chlorinated hydrocarbons, phosphates, mycotoxins and heavy metals. Under feed law, maximum permissible levels apply for the undesirable substances, which are listed in Annex I of Directive 2002/32/EC.

Further demands regarding the reduction of maximum concentrations of individual undesirable substances require consultation and contractual agreement between the consumer and the feed producer.

2.10. Prohibited materials

(Regulation (EC) No 767/2009 on the placing on the market and use of feed)

Annex III of Regulation (EC) No 767/2009 lists the substances that may not be placed on the market as feed even after treatment and/or processing, e.g. faeces, urine, treated seeds, waste or sewage sludge.

2.11. Minimum storage life

(Regulation (EC) No 767/2009 on the placing on the market and use of feed)

The minimum storage life is defined as the “period during which, under proper storage conditions, (…) the feed retains its declared properties”. If this period is exceeded, an analysis can provide information on the further usability of the feed. In the case of purified feeds (see below) or high-fat feeds (fat content ≥10%) and medicated feeds, however, a use-by date or expiry date is specified, which must not be exceeded.

3. Declaration

3.1. Open/semi-open/closed declaration

In the feed declaration, a distinction is made between the (semi-)open and closed declaration: in the open and semi-open declaration, all raw materials present in the feed are listed in descending order by weight. This is required by law in the case of feed for animals intended for food production (animals kept for farming purposes). In the open declaration (“open formula”), the percentages by weight of the respective raw materials are also specified. (Hill et al. 1977, Tobin et al. 2007).

Feed for pets and laboratory animals, on the other hand, often comes with a closed declaration (“closed formula”), in which the feed materials used are grouped together in the group designations required by the German Feed Regulations (e.g. “cereals”, “plant-based by-products”).

3.2. Mandatory information

The following minimum particulars must be included in the labelling for compound feed (according to Article 15 of Regulation (EC) No 767/2009 on the placing on the market and use of feed):

- Producer (with contact details)
- Product name
- Designation of feed type (complete or complementary feed)
- Target animal species
- Composition (raw materials in open, semi-open or closed declaration)
- Ingredients/nutrient content
- Additives (if present)
- Batch number
- Minimum storage life
- Storage instructions
- Feeding instructions
- Net mass

4. Formulas

The formula of a compound feed specifies the types and percentages of all the raw materials used in the feed. The nutrient composition (ingredients) which results from the composition of the raw materials used is to be distinguished from the formula. As regards the variability of a formula, a distinction is drawn between the “fixed formula”, the “semi-fixed/variable formula” and the “least-cost formula”. In the production of laboratory animal feeds, the principle of the fixed formula is preferred for reasons of standardization. The semi-fixed formula is used less frequently. (Tobin et al. 2007).

4.1. Fixed formula

Here, neither the raw materials used nor their percentage content in the formula are changed; this means the formula always remains exactly the same in its raw material composition from batch to batch. To avoid fluctuations in the nutrient composition of the end product, the nutrient contents of the individual raw materials must therefore be as stable as possible. The aim is to standardize both the nutrient composition and the raw materials used (Tobin et al. 2007).

4.2. Semi-fixed/variable formula

In the semi-fixed formula, the raw materials used are also fixed, but their percentage content in the formula may be varied slightly. The aim of this is to counterbalance nutrient fluctuations in the raw materials and thereby prevent any deviation of the end product from the nutrient content (Tobin et al. 2007).

4.3. Least-cost formula

With the least-cost formula, both the raw materials used and their percentage content may be varied on a daily basis so that the desired nutrient contents in the feed are achieved at minimum raw material costs. This principle is mainly used in agricultural feed mills for the production of livestock feed and is not suitable for the feeding of laboratory animal in view of the need for standardization (Tobin et al. 2007).

5. Laboratory animal feed

In laboratory animal nutrition, a distinction is drawn between three types of complete feed: complete feeds based on (1) natural raw materials, (2) purified raw materials and (3) chemically defined raw materials (for summary overview, see Table 1). In German, the term “Diät” is often used in this context, which is a Germanisation of the English term “diet”. This is to be clearly distinguished from the otherwise common German terms “Diät halten” and “Diätfuttermittel” (= feed for special nutritional purposes) according to feed law (Regulation (EC) No 767/2009).

Note: when indicating protein, fat and carbohydrate content, a distinction must be made between the absolute nutrient content and the percentage of the nutrient in the energy content. For example, in the case of high-fat feeds, the percentage of the total energy content that is derived from the fat is often specified. This is not the same as the fat content in percent.

Example: A high-fat feed with 60% energy from fat (“60% kcal fat”) has a fat content (percentage by weight) of around 35%.

5.1. Compound feed based on natural raw materials

(Synonymously used terms: natural ingredient-based diets / cereal-based feed / standard diets / standard feed / chow diet).

5.1.1. Definition

These are complete feeds made from naturally grown raw materials (Hill et al. 1977). The main component is usually a variety of cereals such as wheat or maize, and in the case of rabbit and guinea pig feed also lucerne or hay meal. These feeds must meet the nutrient requirements of the given laboratory animal species in the different phases of life (e.g. reproduction or housing) as best as possible.

These feeds are standardized in that they are produced according to a largely constant (fixed or semi-fixed) formula - i.e. the formulas may only be changed to compensate for native fluctuations. These fluctuations are unavoidable when using naturally grown raw materials such as cereals and are within manageable limits. Fluctuations may also occur due to the use of inconsistent storage conditions by the consumer (see Tables 2 and 3).

5.1.2. Possible uses

Complete feeds based on natural raw materials are offered for various animal species. They are used in the housing and breeding of laboratory animals and in experiments in which the feed is not an explicit component of the study design but only has to be standardized.

The mixing of a substance in these feeds for toxicological or pharmacological studies is possible in principle (see below).

5.2. Compound feed based on purified raw materials

(Synonymously used terms: purified feed / purified diets)

5.2.1. Definition

Purified feeds consist not of naturally grown raw materials, but of purified nutrient components (Hill et al. 1977). In principle, all nutrient fractions required are added to the feed individually, e.g. purified casein as a source of protein, sunflower oil as a source of fat, sucrose or glucose as a source of carbohydrates and cellulose as a source of raw fibre. But even the purified raw materials mentioned here are usually not pure substances but often contain traces of minerals and/or vitamins. Nevertheless, they offer a much better alternative for producing standardized feed than 5.1. The feed can then be specifically modified for certain nutritional experiments, so that an appropriate special feed can be formulated for each research project. Depending on the composition of the feed in question, there may be a reduction in palatability, and this may lead to acceptance problems in the animals. For better acceptance and tolerance, attention should also be paid at all times to making sure that any change of feed is gradual with a corresponding period of time for adaptation. Both apply to purified feed and to chemically defined feed (see 5.3).

5.2.2. Possible uses

Purified feeds have a largely defined and reproducible nutrient composition, which can be varied for specific purposes. Each individual nutrient content can be adjusted depending on the research project. These feeds are therefore used for special feeding-associated experiments, such as the exact measurement of nutrient requirements, the creation of excess or deficiency states, feeding-induced disease models (obesity, diabetes, atherosclerosis) or special toxicological questions. Purified feeds are also particularly suitable for mixing in test substances (see chapter 7).

5.3. Complete feed based on chemically defined raw materials

(Synonymously used terms: chemically defined feed / chemically defined diets)

5.3.1. Definition

Feed based on chemically defined raw materials goes one step further than purified feed: it consists wholly or partially of chemically pure, isolated individual nutrients (Hill et al. 1977). For example, feeds with a defined amino acid spectrum can be produced by dispensing with casein (which contains various amino acids and also traces of minerals and vitamins) as a source of protein and instead adding each individual amino acid in the desired amount.

5.3.2. Possible uses

Chemically defined feeds are similar in use to purified feeds. They are used, for example, when a feed with a defined fatty acid, amino acid or vitamin profile is needed, e.g. to produce symptoms of deficiency or excess. Completely defined feeds from individual chemical components are fully standardized and reproducible, but also very expensive.

6. The production of laboratory animal feed

The quality of laboratory animal feed is determined primarily by the quality of the raw materials and the manufacturing process.

For this reason, quality assurance starts with the product-specific testing of the raw materials to be used.

The criteria applicable to pet food and livestock feed have different points of focus when it comes to the selection of components:

In the case of laboratory animal feed, the focus is primarily on needs-based nutrition, standardization, reproducibility (see fixed formula) and minimization of undesirable influences on the experiment. In the case of pet and livestock feed, consumer demands and market trends always come into play. It is therefore recommended not to use pet and livestock feed for feeding laboratory animals.

Both the manufacturer and the user of laboratory animal feed can help in the standardization of feed use by applying certain measures (see Tables 2 and 3 for examples).

6.1. Manufacturing processes for laboratory animal feed

With regard to the form of the end product, a distinction is made between feed in meal form, pelleted feed, extruded feed and expanded feed.

The technological production processes are largely identical to those used for livestock feed and pet food. In the production of laboratory animal feed, however, the production batches are much smaller, because the production volume is significantly lower and the specific requirements of the laboratory animal facilities have to be taken into account. This means that the production line needs to be cleaned after the production of species-specific feeds. When it comes to pelleting, the shaping die (= metal mould containing the compression channels; see Fig.1) is changed frequently due to different pellet sizes.

Growing requirements of compound feed quality - such as increasing nutrient availability without damaging proteins and sensitive additives (e.g. vitamins) or reducing germs - have led to the development of new technological processes, especially with regard to hydrothermal treatment and mechanical loading (see Tables 4 and 5). In all production processes, possible heat-related nutrient losses are already offset by the manufacturer in the formulation.

6.2. Feed in the form of meal

The use of complete feeds in meal form in laboratory animal facilities is reserved for special research issues (e.g. metabolic cage, administration of thermolabile substances via the feed) because they have various disadvantages compared with compressed feeds, e.g.:

- reduced homogeneity as a result of separation
- reduced hygienic stability as a result of increased surface area
- more difficult feeding technique, because the meal cannot be administered through wire racks

For the production of laboratory animal feed, meal-type feeds are generally first pelleted and then ground. This increases the homogeneity of the mixture, prevents separation and hence selective feed intake and reduces the germ count by heating. A rare exception to this are special feeds in meal form, whose ingredients (e.g. test substances) would suffer heat damage if pelleted. In this case, the components are simply mixed homogeneously to form a meal feed.

6.3. Pelleted feed

Compared with compound feeds in meal form, pelleted feeds offer advantages in transport, in handling by the animal keeper and for the animal itself:

- free-flowing, dosable pellets
- no separation during bulk transport and silo filling

- improved storability
- only minimal feed losses due to scattering
- increased storage stability thanks to reduced microbial burden
- no selective intake of components by the animal
- high quality and stability of hygiene
- problem-free feeding via cage racks (mouse, rat)
- better acceptance
- gnawing activity is possible

Before pelleting, the feed mixture is steam-treated (conditioning). This optimizes the subsequent compression process with regard to both energy input and pellet strength. The required steam is generated in a pressure vessel and is the only medium through which thermal energy is transferred to the feed mixture. An additional thermal influence then arises during the compression process as a result of the frictional heat in the die.

In the compression process itself, the feed mixture is pressed into the channels of a shaping die by means of rotating ring travellers (rollers) (Fig. 1): the meal evenly fed into the die forms a layer, and the rollers compress this layer as they roll over it and press it into the individual compression channels. With each rollover, a new layer of feed is pressed into the channel. The pressure increases until the feed column in the press channel starts to move.

Knives on the outside of the die break off the columns of feed that are pressed through the channels into cylindrical pieces, i.e. the pellets. On exiting the die, the length of the pellet is usually 1.5 to 2 times the diameter. Customary pellet diameters are 2, 3, 4, 10, 12 and 15 mm.

The pellets leaving the press cannot be stored straightaway because of their residual moisture and core temperature. So they must always be cooled and dried to ambient temperature immediately after leaving the press. Insufficient cooling means reheating starting from the pellet core and carries the risk of the feed being spoiled.

6.3.1. Effects of pelleting process on additives and feed hygiene

The initial steam treatment of the feed mixture briefly exposes the nutrients contained in the feed to high temperatures that have the potential to damage the nutrients. During the compression process in the die, temperatures of up to max. 80°C also occur briefly in the outer layers of the pellets, exposing the added nutrients here to thermal risks and alteration. This loss is already taken into account and offset by the manufacturers when formulating the feed.

However, the thermal influence during the pelleting of compound feeds also has a positive effect: the prior steam conditioning reduces the microbial burden of the compound feed.

6.3.2. Effects on compressibility of the feed mixture

Raw materials and the formulations composed of these materials have diverse and not always unequivocally predictable physical properties. A raw material may behave differently in combination with other raw materials, so the raw material and formulation must be assessed as a unit.

The overall properties of a formula are affected by

1. the characteristics of the chemical substances (ingredients) and their mix ratios
   - Protein content
   - Fibre content
   - Fat content
   - Ash content (minerals)
   - Sugar content
2. the physical characteristics
   - Structure (particle size distribution, particle shape)
   - Moisture
   - Homogeneity
3. the properties of additives (compression additives)
   - Adhesive, lubricant, inhibitory
4. the technology used
   - for comminution (milling, degree of fineness)
   - for conditioning (temperature, moisture, time)
   - for compression (die dimensions, rollers, throughput)
   - for cooling (temperature, air volume, time)

Finely structured mixes achieve better pellet quality than coarse milling processes.

Coarsely milled mixes generally lead to poorer pellet quality, as the coarse individual particles cause the pellets to break easily. This also results in increased abrasion and poorer homogeneity.

The raw materials used in natural feeds are predominantly natural products, which are always subject to fluctuations in their properties. These fluctuations are one reason for possible variations in pellet quality. Influences resulting from ingredients can therefore only be incompletely described.

6.3.3. Influences of ingredients on pellet quality

<u>Protein content</u>:

An increase in protein content (from approx. 25% upwards) leads to harder pellets. Compression resistance increases slightly.

<u>Fibre content</u>:

In general, raw fibre (from approx. 10% upwards) increases friction and makes the pellets harder. Compression resistance is greater with increasing fibre content than with other ingredients.

Fat content:

The higher the fat content in a mixture, the softer the pellets become. Pelletability therefore falls with increasing fat content. At very high fat contents (from approx. 40% crude fat upwards), pelleting is almost impossible. The feed can then only be produced in paste form.

Ash content:

The addition of inorganic components (e.g. minerals) results in harder pellets.

Sugar and starch content:

Freely added quantities of sugar or starch lead to very solid pellets, as the sugar caramelizes and the starch gelatinizes under the influence of the temperature during pelleting.

6.4. Extruded and expanded feeds

Pellet feeding is not appropriate for all laboratory animal species, because some species (e.g. carnivores, primates) show a reduced acceptance of pellets. In these cases, extruded or expanded feed is used.

An extruder consists of one or two screw shafts that rotate in a housing and convey the mixture to be extruded against a pressure differential. After steam is added, the feed mixture is subjected to mixing, shearing and kneading forces, dissipative heat, increasing compression and a rise in pressure. Finally, a shaping process takes place by means of a shaping nozzle.

In contrast to pelleting, temperatures significantly higher than 100°C are reached for a short time. The pressure and temperature are at their highest just before the mixture leaves the shaping nozzle (die). The higher temperatures combined with a significantly higher pressure enables significantly better hygienisation of the end product to be achieved with expansion/extrusion than with pelleting.

Aside from the normal housing, the characteristic elements of an extruder are (Fig. 2):

- variable configurations of the screw shafts
- replaceable shaping nozzle(s)/die(s) with aperture of defined shape and size
- double jacket for indirect cooling or heating
- Injectors for feeding steam or liquids into the process chamber under positive pressure.

Extrusion is a highly flexible process and suitable for a variety of raw materials, variable treatment conditions or the manufacture of different product shapes.

The end product (extrudate) exits through a shaping die and must then be dried and cooled to ensure storage stability. The flow behaviour of extrudates is comparable with that of pellets. The bulk density is lower than that of pellets.

An expander can be regarded as a simplified and low-cost extruder. Unlike most extruders, an expander is a single-shaft machine with a fixed screw configuration (Fig. 3). The expansion of the mix is accompanied by a kneading process, dissipative heating and pressure increase, and a sudden pressure drop (expansion) as it exits the annular die. Unlike extrusion, expanding does not produce a defined shape for the treated material. Expanders are mainly integrated into existing production lines as pressure conditioners between the short-term conditioner and the pellet press. Expansion essentially results in qualitative effects.

Effects of the expander and extruder:

- Reduction of germ count (greater than with pelleting)
- Digestion of starch (increased palatability and digestibility)
- Degradation of thermolabile antinutritive substances
- Possible damage to additives: e.g. vitamins, amino acids (formation of condensation products)

6.5. Reduction of germ count in various production processes

The germ count is reduced by the effect of the heat during the production process. This effect is significantly greater with extrusion than with pelleting as a result of higher temperatures and higher pressure. A reduction of the germ count always occurs exponentially; so the possible rate of decontamination is indicated in powers of ten. The result depends, among other things, on the initial microbial burden of the individual raw materials, the treatment intensity (temperature and humidity), the treatment time and the structure of the feed (particle size). After production, the germ reduction achieved in this way must be protected against recontamination by appropriate measures (drying, cooling, packaging, hygiene regimen).

The germ reduction achieved based on the initial microbial burden of the mix:

- Pelleting: reduction by 10^{-2} to 10^{-3}
- Expansion and extrusion: reduction by 10^{-5} to 10^{-7}

7. Incorporation of substances into laboratory animal feed

Substances can also be added to the above-mentioned laboratory animal feeds. In pharmacology, toxicology and in chemically induced transgenic models, the oral administration of substances with the feed can be an animal welfare-friendly alternative to administering the substances by gavage or injection. However, the appropriateness of this alternative must be decided on a case-by-case basis. The incorporation of substances requires intensive coordination between the experimenter and the producer, in which the following points must be observed:

1. Choice of basic feed: for reasons of homogeneity and standardization, it is generally recommended that purified feed be sued for the incorporation of test substances. The investigator must always weigh up which is worse: the possibility of poorer substance distribution in a natural feed or the change of feed when using a purified feed.
2. The stability of the substance vis-à-vis process-related influences (pressure, temperature, humidity): the usual form of feed for the incorporation of substance is the pellet. Pelleting generates frictional heat, which should not affect the substance to be mixed in. In the case of thermally labile substances, it is therefore only possible to mix them into a meal-type feed. Extrusion is not generally used because of the high temperatures.

3. Chemical and physical properties of substance and basic feed: density, particle size distribution, structure, miscibility, homogeneity, stability, analytics, occupational safety aspects.

Basic composition, presentation form and consistency should be identical for both control and experimental feed. The risk of confusing these feeds in the study can be avoided by using conspicuously different packaging or, if possible, by incorporating colouring additives.

8. Shipment

8.1. Consignments of loose feed

Consignments of loose feed require suitable storage containers in the animal facility, e.g. silo cells. The following must be observed:

- Only indoor silos should be used for laboratory animal feed (reduction of temperature fluctuations, less risk of infestation with pests).
- Only flawless connection pieces must be used to blow out the transport container, so that no impact surfaces are created. The blow-out pressure must be adapted to take account of the product’s consistency.
- Feed residues in blind corners must be regularly removed.
- Thorough emptying with subsequent cleaning and disinfection must be carried out on a regular basis.

8.2. Packaging

Non-sterilized laboratory animal feed for conventional animal facilities is usually packed in paper bags, which should have at least a triple layer of paper.

Feed intended for autoclaving is supplied in perforated paper bags so the steam can reach the feed. To prevent the formation of lumps, feed intended for autoclaving may be dusted with powder (nutritionally neutral silicates/plasticizers) immediately before bagging.

Low microbial feed or feed intended for irradiation is packed in sealed plastic bags or in paper bags with plastic wrapping. Vacuum packaging is also useful.

For transport, the feed bags are stacked evenly on dry, clean pallets and wrapped in film. The type of pallet must be adapted to the hygienic requirements of the animal facility concerned. For example, pallets made of plastic or metal are common, as are heat-treated wooden pallets. After acceptance in the feed store, the outer film must be removed immediately so that the breathability of the feed is not impaired (risk of condensation and mould). During storage, the points listed in Table 3 should always be observed.

9. Annex

9.1. Tables

Table 1: Summary overview: complete feed - components, uses and possibilities for standardization

Description	International designation	Components	Use	Standardization	Limitations
Complete feed based on natural raw materials	**Natural ingredient-based diet**	Plant-based and animal-based feed materials, mineral and vitamin premixes	Breeding and husbandry, substance incorporation	Given	Limited variability and reproducibility especially in experiments
Complete feed based on purified materials	**Purified diet**	Isolated protein, carbohydrate, fat and oil fractions, mineral and vitamin premixes	Induction of deficiency and excess symptoms, substance incorporation	Good	High costs, acceptance problems (adaptation!)
Complete feed based on chemically defined raw materials	**Chemically defined diet**	Isolated amino acids, mono/disaccharides, fatty acids, minerals and vitamins if high purity, additives	Induction of deficiency and excess symptoms	Very good	Very high costs, acceptance problems (adaptation!), requires very precise knowledge of needs

Table 2: Focus of standardization efforts for laboratory animal feed by the producer.

Factor	**Contribution to standardization**
Raw material selection	• Suitability for animal species in question • Constancy of nutrient composition of the raw materials • Compliance with upper limits for undesirable substances
Formula + nutrient composition	• Fixed formula
Hygiene quality	• High level of hygiene, free from pests • Reduction of microbial count and species • Optimum storability • Irradiation

Table 3: Focus of standardization efforts for laboratory animal feed by the consumer

Factor	**Contribution to standardization**
Storage Standard feed	• rooms closed on all sides, cool and easy to clean • smooth walls and floors free of cracks • good ventilation • FIFO (first-in-first-out) principle • tightly sealed windows and doors (e.g. double-door airlocks) • rodent barriers, pest control • gauze windows • temperature in long-term storage: ideally not higher than Central European room temperature[1) 2)] • relative humidity: <70%[1) 2)] • avoid major temperature fluctuations2) • storage time as short as possible, max. by end of MHD
Feed treatment for sterilization	• autoclaving

[1)] The data given here constitute the lowest common denominator from several publications (Clarke et al. 1977, Fullerton et al. 1982, Tobin et al. 2007) and recommendations of producers.

[2)] Cooling of the storage rooms for standard feed is not absolutely essential. Humidity has a greater influence on the storability of standard feed than temperature. Short-term storage above normal room temperature is generally not a problem. Rapid and marked temperature fluctuations must be avoided at all costs, as this gives rise to condensation, which causes feed spoilage.

9.2. Figures

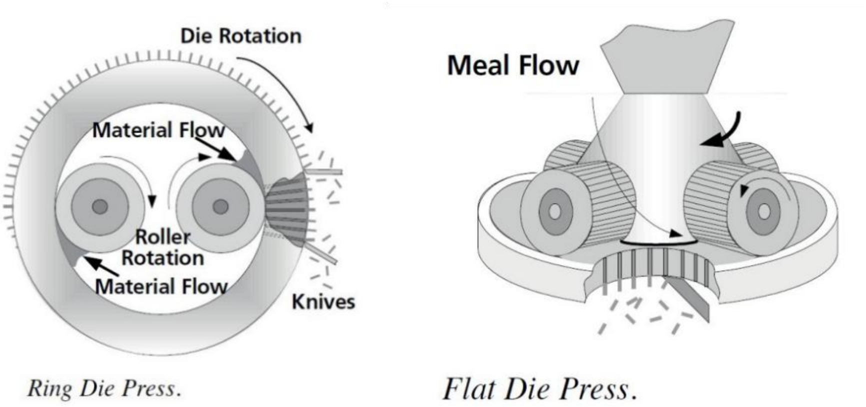

Figure 1: Pellet press: shaping die with rollers (Borregaard Ligno Tech, 2016)

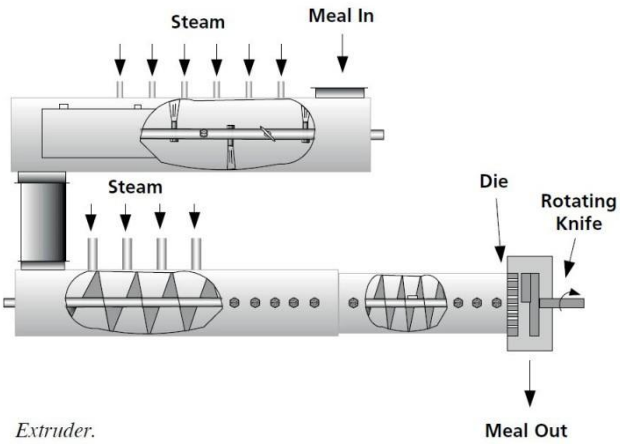

Figure 2: Extruder (Borregaard Ligno Tech, 2016)

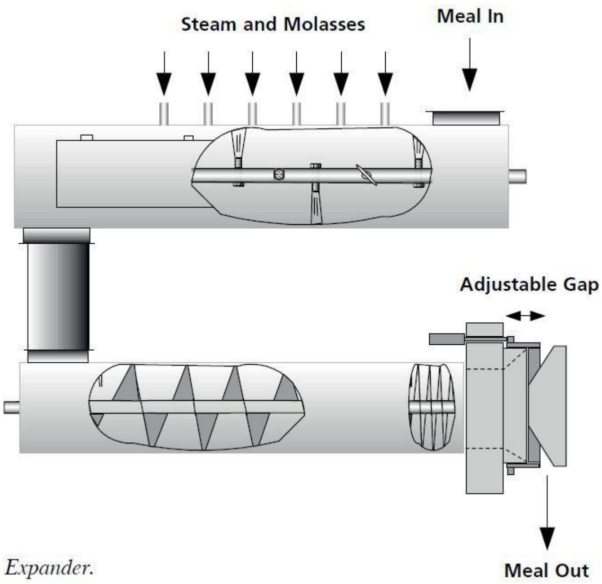

Figure 3: Expander (Borregaard Ligno Tech, 2016)

10. References

Laws and regulations

1. Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (consolidated; 1 July 2018);
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