Society of Nutrition Physiology (GfE)

Opinion on the indispensability of animal experiments in animal nutrition research and suitability of alternative methods

Approved translation of the original version published in German:
Gesellschaft für Ernährungsphysiologie (2017): Stellungnahme zur Unerlässlichkeit von Tierversuchen und zur Eignung von Ersatzmethoden in der Tierernährungsforschung. Proc. Soc. Nutr. Physiol. 26:218-224

Synopsis

The public has high expectations regarding availability of food, including global food security, feed and food safety, animal welfare, and the protection of natural resources and of the environment. To meet these expectations and solve conflicts of interest requires extensive research. Animal nutrition research needs both, animal trials and alternative replacement methods. If appropriate alternative methods are not available, animal trials are indispensable. The planning and carrying out of animal trials are based on the 3 R principle, minimizing any load, stress and discomfort of the animals.

The keeping and use of animals by humans are an integral part of social activity and subject of public discussion. Different perceptions for farm animal husbandry therefore have consequences for the scientific work with animals and for the framework conditions under which research activity develops.

Animal experiments are critically judged by parts of the society or completely opposed. However, despite all the successes in the development and establishment of alternative non-animal approaches, animal experiments are still indispensable. The explanation and justification of indispensability is a prerequisite for animal experiments to be accepted by the society. The reasons go beyond the intrinsic motivation and scientific responsibility of the researchers as well as the legal mandate for scientific research. The indispensability of animal experiments is determined by the manifold demands and expectations which are the result of complex social discussions and the resulting political decisions.

The aim of the present opinion is to provide explanations for the necessity of experimental studies with animals in animal nutrition research. To this end, the tasks of animal nutrition are explained first. It will then be shown which methods are common in animal nutrition research and how these methods also enable the development and improvement of alternative methods. Science-based comments on animal experiments are also provided, for example, by the Senate Commission on Animal Protection and Experimentation of the German Research Foundation[1] and the Max Planck Society[2].

[1] The Senate Commission on Animal Protection and Experimentation (ed.): Animal experimentation in research. http://www.dfg.de/en/dfg_profile/statutory_bodies/senate/animal_protection/index.html. Accessed 21st January 2017.
[2] White paper on animal research of the Max Planck Society. https://www.mpg.de/10930951/white-paper-animal-research. Accessed 21st January 2017.

1. Framework and tasks of animal nutrition research

Farm animals are an integral part of agricultural systems, nutrient cycles and the environment. All scenarios for the future global population development are characterized by a high demand for food of animal origin (milk, meat, eggs and fish, as well as their respective processed products). If this demand is to be covered, a set of measures is required that includes the reduction of losses along the production and processing chain, but also promotes the science-based development of livestock systems. The production of plant- and animal-derived food is already limited by the size of the agricultural area globally available, of which the predominant part is permanent grassland. Furthermore, the production of primary agricultural products depends on the availability of water and nutrient resources that are globally scarce and unequally distributed, some of which are finite. In this regard, **food security** for the growing world population is one of the major challenges for the future.

Food should be available not only in sufficient quantities and at affordable prices, but should also provide high quality and safety for the consumer. Food safety begins with the safety of the feed, because the composition of animal derived food is influenced by the animal's feeding. This applies to beneficial constituents as well as to undesirable substances or some pathogens which represent a potential hazard for humans. Knowledge about a possible transfer of substances and pathogens from the feed via the animal to the food is therefore indispensable for the **assessment of food safety.** Thus, the legal framework requires animal experiments if, for example, feed additives are evaluated for authorization.

The metabolism of humans and animals has biological limits, which inevitably entail losses. Many feed constituents cannot be fully utilised, which leads to the excretion via faeces and urine of the animals, the release of gases and the formation of heat. If the stocking density of animals in relation to arable land in a region is high and arable land is scarce, an accumulation of nutrients and undesirable entries of, for example, nitrate and phosphate in soils and water bodies through manure application are possible. Emission of gases such as ammonia and methane associated with animal husbandry also may affect the environment. The society rightly expects that **negative effects of animal husbandry on the environment** are minimised as far as possible.

Established forms of animal husbandry are critically questioned by parts of the society in Germany with regard to the adequacy of production conditions, sometimes even rejected. **Animal health and well-being** are of high value. However, actions taken to maintain health and enable species-specific animal behaviour like a high supply of forage to stimulate rumination in cattle can have a negative impact on the environment and the productivity of animal husbandry. Such **conflicts of interests** can only be solved or at least reduced by the society in a discursive process that is based on facts and science-based arguments for meaningful actions to be taken in the future.

In the context of global food security, feed and food safety as well as animal and environmental protection, **animal nutrition has a predominant role**. First of all, adequate nutrient supply of the animals is crucial to avoid deficiencies and diseases, and essential for welfare and productivity. With targeted feeding, efforts are also being made to improve nutrient utilisation and thus to reduce their excretion and their impact on the environment. Feeds and feeding practice also influence the contents of nutrients and sensory criteria in products such as meat, milk and eggs, and consequently also affect the processing properties of these products.

In order to be able to supply animals with all nutrients in accordance with their needs, the feedstuff base must be reconciled with the requirements of the animals. However, both segments, the feedstuff base and the demands of the animals are variable and the latter is constantly changing. On the one hand, there is a certain, genetically determined performance potential of the animal, which is different between species, breeds, lines and target of use. On the other hand, the feedstuff base is also subject to change, for example due to plant breeding activities or changes in agronomic and environmental conditions. By-products resulting from the processing of food (e.g. bran or middling), which play a

major role in the feed sector, are changing not least by advances in technological processes. In addition, certain feedstuffs such as imported soybean, are unwanted by retailers and consumers -irrespective of objective assessments of feed safety- and are therefore to be replaced by alternatives. In the field of feed additives, such as enzymes or preservatives, new products are constantly being developed that need to be investigated with respect to their efficacy. The challenges arising from this dynamic in the two segments and the underlying metabolic processes are the central areas of work in animal nutrition research. The areas range from applied animal feeding towards nutritional physiology and basic biological aspects of the animal's metabolism.

Research in the field of animal nutrition is diversified according to the framework conditions outlined above, and must be carried out with the various animal species and fields of use, also considering the age of the animal. The research activities can be grouped into the following **thematic areas**:

- Studies on the development of the animal, its body composition, behaviour and well-being, as well as its digestive and metabolic processes, taking into account animal and environmental influences;
- Quantification of the transfer of desired and undesirable substances and pathogens into the animal, its products and their excretions;
- Identification of requirements and derivation of supply recommendations to ensure the health and performance of different species and production targets;
- Studies on the consequences of diets on metabolic disorders, illness or malnutrition, and the development of preventive measures;
- Evaluation of new and modified feedstuffs and feed additives as well as further development of the methodological repertoire, including the development of alternative non-animal approaches.

In order to answer many questions within the scope of the tasks outlined above, the implementation of animal experiments is indispensable. Alternative methods which do not require any or a considerably reduced number of animals are also carried out and preferred if they are sufficiently precise. But for certain questions, no adequate alternatives exist yet, such as the transfer of feed constituents into animal-derived products. An important objective of animal nutrition research therefore is to further develop and validate non-animal approaches in order to permanently reduce the use of animals for scientific purposes. Animal experiments in animal nutrition research are only carried out if they are indispensable for answering the respective specific question. However, it must be kept in mind that animal experiments are indispensable for the development and calibration of alternative approaches.

2. Methods and techniques used in animal nutrition experiments

Animal experiments conducted within the framework of feed evaluation and nutritional physiology deal with feeds and feeding and their effects on the animal, the product obtained from them, and the environment. They comprise a wide range of methods and techniques, the selection of which depends on the specific research objective. In principle such procedures are selected or developed that promise to give a reliable answer to the question. The various approaches can be grouped into five blocks, as outlined below. In experiments, methods from two or more of these blocks can also be combined.

a. Feeding trials

Feeding trials are designed to investigate how different feeds, additives or feed processing techniques may affect the feed intake of the animals, health of the animal and its specific organs, performance criteria such as growth, milk or egg yield, the quality characteristics of the products obtained or other criteria, such as animal behaviour. These trials may require adjusted housing conditions or interference with the animal (also see e.). Depending on the research question, information, findings and data are also collected only after animals were slaughtered, or animals

for the examination of organs and tissues are killed in order to detect influences on the health or transfer of substances and pathogens into the animals, their organs and products.

b. Digestibility trials

Digestibility data are the basis of any rational feed evaluation. In digestibility trials, the proportion of feed constituents recovered in the faeces is measured in order to calculate the part of the feed that is available to the metabolism of the animal. The standard measurement of digestibility requires that the amount of feed consumed and the amount of faeces are precisely measured in each individual animal. For this purpose, suitable housing conditions are necessary in which the faeces can be collected **without any losses**. This implies that the freedom of movement of the animals and the contact between them are considerably limited. Under certain circumstances faeces can alternatively be collected by using bags or harnesses attached to the animal; however, this might lead to incomplete faeces collection. The animal has more movement and contact possibilities this way, but a certain limitation of moving activity still exists. A digestibility test can also be carried out using special markers combined with spot sampling of the faeces. A complete collection of faeces is not necessary here. However, the accuracy of the data acquisition when using markers is lower than in the case of a complete collection of faeces, which leads to a larger number of animals required in the trial. Of note, the suitability of a marker can be assessed only in experiments with complete collection of feed and faeces.

With the collection and analysis of the faeces alone, however, processes in individual sections of the digestive tract (e.g., the rumen or small intestine) cannot be investigated more closely. Studies in specific compartments of the digestive tract are, however, very important or even decisive for many questions of animal nutrition research, e.g. the importance of the rumen for animal health. Surgical interventions (also see point e.) give access to the digestive tract section of interest (‘fistulation’), which allows the regular withdrawal of material from or input in the digestive tract during the experiment. These techniques gained particular importance in the development of in vitro simulation techniques (e.g., for the rumen of ruminants or the caecum of horses). In this case, the produced metabolites are not measured in the animal, but in samples taken from the digestive tract, which are then further processed under defined conditions outside the animal (in vitro). In this way, a better understanding of transformations in certain parts of the digestive tract can be achieved. Furthermore, digestive processes may be studied by taking material from the digestive tract immediately after slaughtering the animals, thus avoiding any treatment of the alive animal.

c. Balance trials

Research questions which, in addition to processes in the digestive tract, are also related to the metabolism beyond the intestinal wall, require so-called balance trials. In balance trials, in addition to faeces, the excretion of substances via the urine is also measured. The housing condition therefore must allow a complete and separate collection of both, faeces and urine and hence requires a considerable restriction on the possibilities of the animal to move freely. Markers, which allow the determination of the amount of urine similar to faecal markers, are not yet available. In balance trials, products (e.g., milk or eggs) must also be recorded separately for each individual animal. The fractions accreted in the body of the animal can be calculated from the data of the balance trial, if no gaseous losses occur. Alternatively, the accretion of a substance can also be determined by way of a comparative whole body analysis, in which the animals are slaughtered in predetermined growth intervals following a defined feeding. In such studies, the proportion of undesired substances such as toxins or heavy metals and their transfer rates into the body or specific organs can also be determined.

d. Respiration trials

Respiration trials represent an extension of balance trials in which the consumption of oxygen and the release of gases (carbon dioxide and methane) are additionally measured. These gas exchange measurements are particularly important for tracing changes in body composition and energy

accretion of the animals. This information is the indispensable basis for all international systems used for the energetic feed evaluation. Furthermore, the quantification of, in particular, methane release makes it possible to assess effects of the animal on the environment. The complete measurement of the gases exceeds the technical possibilities of the aforementioned balance trials because both the volumes and the composition of the inhaled and released gases must be determined continuously. In the reference method, this is done in closed, gas-tight compartments in which both the supply and exhaust air flows are recorded without any losses. Only a few research institutions worldwide have access to this sophisticated technology. For this reason, methods for estimating the methane release with larger numbers of animals and without the need for fixation (e.g., infrared absorption techniques or tracer gases) are therefore used – especially for the determination of methane release in cattle. These estimation methods have to be calibrated before and in parallel to results from respiration trials, but are nevertheless associated with considerable intrinsic inaccuracies.

e. Other experimental techniques

Other techniques, frequently used in combination with the trial techniques mentioned before, are blood sampling by venous puncture and the separate discharge and collection of the urine. The specific function of an organ or secretions of an organ can be studied by setting apart a specific organ or by draining secretes (e.g., saliva). In addition, tissue and organ samples can be obtained from the living animal (biopsies of the liver, adipose tissue and muscles under anesthesia or in narcosis, respectively) in order to examine e.g. allergic reactions or metabolic processes. Finally, there are also trials in which the relevance of the feed or feed additives for the course of infections in a herd is examined. These experiments are particularly relevant if infections are concerned which can transmit from animals to humans (so-called zoonoses, e.g., salmonellosis). To investigate the pathways of infections and the efficacy of measures to control transmission, the pathogens are administered to individual animals so that they act as 'seeder' in an animal group. Such experiments are of paramount importance when suitable preventive feeding measures are developed to be used for improved food safety by avoiding human infection.

3. Assessment of the methods and approaches to reduce the number and stress of animals

Animal trials are very complex and must therefore begin with careful planning taking into account the national and European legal framework. The planning involves clarifying whether the experiment is scientifically justified and indispensable, and whether non-animal approaches are available. Animal trials are only justifiable if suitable alternative methods are not available and the animal trial is therefore indispensable.

Alternative methods in animal nutrition research range from purely analytical methods in the laboratory through in vitro experiments and cell culture or tissue studies to computer simulations. These alternative methods are intended to depict processes in organs, animals or a herd as good as possible. They should allow routine use and must be sufficiently accurate. Therefore, they can only be generated, derived and regularly validated using data from animal trials. The development and improvement of alternative methods is thus an integral part of scientific work in animal nutrition. Alternative methods, however, can only partially depict the complex biological reality of an entire organism and are always subject to uncertainties and errors. When planning trials, it is important to decide in each individual research case whether the error of a method is acceptable for the specific research question and a non-animal approach can therefore be used or whether the accuracy required makes an animal trial indispensable.

If an animal trial is found to be indispensable and planned, the aim is to reduce the number of animals to a minimum. For this purpose, a biometric planning is performed by using preliminary information regarding variation and magnitude of any treatment effect. The number of animals is determined in such a way that a treatment effect, if present, can actually be demonstrated in the experiment. Guidelines

for the planning and implementation of certain animal trials, such as digestibility trials, have been developed by scientific committees. These guidelines also established minimum values for the number of animals to be used for the purpose.

In the case of digestibility, balance and respiration trials, the restriction of free movement and contact might be a stress factor to the animal. Stress can be accepted in consideration of all arguments if there is no alternative to answer the question. In many cases, trials using a small number of animals will yield insights that provide significant benefits for large populations of livestock, humans or the environment.

The duration of the restrictions is limited to a minimum. Guidelines for digestibility trials exist which, taking into account the type of animal, specify how long the collection of the faeces must last in order to obtain a representative sample of the faeces. For example, the Society of Nutrition Physiology is currently recommending a period of five days for pigs and a minimum of seven days for sheep, while ongoing projects should clarify whether these periods can be shortened. Reactions of the animal to stressors can be considerably reduced if animals are not kept individually, but in pairs or in small groups, or at least visual contact is ensured. One of the most important measures is the slow and gradual adaptation of the animals to the experimental conditions. The personnel are obliged to spend a considerable amount of time in the animal house with contact to the animals and to provide intensive care to the animals.

Certain research questions in the field of animal nutrition research require the above-mentioned surgical interventions to access the digestive tract in order to be able to obtain gastric or intestinal contents from the living animal. These surgical procedures are performed under appropriate anesthesia or in narcosis, require a medical pain relief for a short time and generally need particular justification. After healing is complete, fistulated animals can be used as ‘donor animals’ for several months or even many years, depending on the animal species. This allows to carry out corresponding in vitro methods, such as the Hohenheim Gas Test, which significantly contribute to the reduction of animal trials.

It currently is under investigation whether or not the time period animals are restricted to move can be further reduced in balance trials, perhaps related to a need for a higher number of animals to be used. In this context, interdisciplinary initiatives are encouraged in which behavioural scientists feel encouraged to identify criteria that reflect the load, stress, or suffering of animals exposed to restricted moving activity, to deprivation of group members, to handling and treatment, including the repeated use of the same animals in subsequent trials.

Authors:
Standing Committee of Requirement Standards of the Society of Nutrition Physiology
Board of Directors of the Society of Nutrition Physiology
Eschborner Landstraße 122
60489 Frankfurt am Main

Members of the Standing Committee:
Kamphues, J., Hannover
Rodehutscord, M., Hohenheim (Chair)
Schenkel, H., Hohenheim
Staudacher, W., Frankfurt
Südekum, K.-H., Bonn
Susenbeth, A., Kiel
Windisch, W., Freising-Weihenstephan

Members of the Board:
Aschenbach, J., Berlin
Dänicke, S., Braunschweig
Metges, C., Dummerstorf
Sauerwein, H., Bonn
Windisch, W., Freising-Weihenstephan (Chair)
Zebeli, Q., Wien