Predicting the Transfer of Contaminants in Ruminants by Models – Potentials and Challenges

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Abstract
Undesirable substances in feed can transfer into foods of animal origin after ingestion by livestock animals. These contaminants in food may threaten consumer health. Commonly, feeding trials are conducted with animals to assess the transfer of undesirable substances into animal tissues or milk. Such feeding trials explore the effects of the various physiological systems (e.g., ruminant and non-ruminant gastro-intestinal tracts) as well as different livestock production intensities on transfer. Using alternative methods to mimic the complex physiological processes of several organs is highly challenging. This review proposes a potential cascade of in vitro and ex vivo models to investigate the transfer of contaminants from feed into foods of animal origin. One distinct challenge regarding the models for ruminants is the simulation of the fore stomach system, with the rumen as the anaerobic fermentation chamber and its epithelial surfaces for absorption. Therefore, emphasis is placed on in vitro systems simulating the rumen with its microbial ecosystem as well as on ex vivo systems to replicate epithelial absorption. Further, the transfer from blood into milk must be evaluated by employing a suitable model. Finally, in silico approaches are introduced that can fill knowledge gaps or substitute in vitro and ex vivo models. Physiologically-based toxicokinetics combines the information gained from all alternative methods to simulate the transfer of ingested undesirable substances into foods of animal origin.

1 Introduction

Expectations of animal-derived food are high. Besides being available in sufficient quantities, animal-derived food is expected to be of high nutritional value, while food safety risks to consumer health must be low. Undesirable substances in feed can pose a threat to consumer health if, upon ingestion by livestock, they are transferred into foods of animal origin, where they are then considered contaminants.

“Undesirable substances” in EU legislation on animal nutrition means any substance or product, except for pathogenic agents, present in and/or on the product intended for animal feed that presents a potential danger to human health, animal health or the environment or adversely affects livestock production (Directive 2002/32/EC). In comparison, “contaminants” are substances that have not been intentionally added to but may be present in food as a result of the various stages of its production, packaging, transport or storage. They also might result from environmental contamination (Council Regulation (EEC) No 315/93). Accordingly, undesirable substances are regulated for feed, while contaminants are regulated for food.

REACH (Regulation (EC) No 1907/2006) requires, according to Article 14(4), exposure assessment and subsequent risk characterization to be carried out for chemical substances subject to registration that are manufactured or imported in quantities equal to or greater than 10 (metric) tons per year and where the substance fulfills the criteria for any of the hazard classes or categories indicated in Article 14(4) or is assessed to be persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB). As such, for substances in plant protection products (Council Directive 91/414/EEC) as well as for the authorization of several feed additives (Commission Regulation (EC) No 429/2008), investigations on a possible transfer from feed to food are mandatory to establish withdrawal periods, maximum residue levels (MRLs) or maximum contents in feed-stuffs (Directive 2002/32/EC). This routinely requires feeding

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trials with various livestock species to cover the different physiological systems (e.g., ruminant gastro-intestinal tract (GIT) and non-ruminant GIT). These in vivo trials are time-consuming, expensive, and often require killing of the animals to harvest organs and tissues for contaminant analyses (e.g., according to OECD TG 503 and OECD TG 505; OECD, 2007a,b). Following the 3R principle (replace, reduce, refine) introduced by Russel and Burch (1959), alternative models (in vitro, ex vivo, in silico) are being developed and tested to simulate the relevant in vivo processes as closely as possible. To employ them for regulatory purposes, their development needs to undergo a thorough validation to ensure scientific integrity and quality (OECD, 2018).

Various partial gastro-intestinal in vitro and ex vivo systems to evaluate the nutritional value of feedstuffs already were established decades ago. These can simulate the process of nutrient digestion and absorption in the GIT. However, so far, they have been used only rarely to study the transfer and metabolism of undesirable substances in the animal. Moreover, to investigate potential substance transfer into animal tissues or excreta when ingested with feed, the physiological processes downstream need to be simulated by a cascade of individual methods to capture the whole complexity of animal metabolism. Simulating processes in the ruminant is especially challenging due to the physiological distinctiveness of the forestomach system and the highly different metabolic statuses the animal can be in during its life, e.g., dairy cows during the onset of lactation vs. beef cattle.

Recently introduced in silico models may help to simulate the interactions between individual body compartments as well as species-specific differences. The decision on whether non-animal methods are suitable for reproducing the metabolism of an undesirable substance in the animal requires a detailed examination of the functioning, potential, and limitations of each individual method (Tab. 1).

The present report reviews in vitro and ex vivo models that simulate the physiological processes in ruminant species, beginning with the complex forestomach system, including intestinal absorption and hepatic metabolism, and ending in excretion via milk. The focus is on the fate of foreign substances in the organism. In this context, the fate is called kinetics and the substances xenobiotics. An in silico approach of kinetic modelling is introduced to model the interactions between the individual in vitro and ex vivo compartments to complete the understanding of the entire animal metabolism and close the gap between in vitro and in vivo results.

2 In vitro reticulo-ruminal digestion and fermentation

2.1 General considerations regarding ruminant species

Ruminants are unique in their mode of plant digestion, as they have evolved a forestomach, which allows microbial digestion and fermentation of fibrous carbohydrates, i.e., cellulososes and hemicelluloses, leading to the formation of short chain fatty acids (SCFA), which constitute the major energy source for the ruminant (Bergman, 1990). The ruminant’s stomach system is composed of the reticulum, rumen, omasum, and abomasum. The reticulum and rumen are joined by a fold of tissue and constitute a large fermentation chamber that houses a highly diverse rumen microbiome with manifold enzymatic activities (Moraes and Mizrahi, 2019). The rumen wall is covered by a keratinizing stratified squamous epithelium in the form of countless papillae for effective absorption and secretion via a permeation barrier (Aschenbach et al., 2019). The reticulum is further connected to the omasum, also called manyplies because of the numerous parallel sheets of tissue, which serves as a bottleneck for feed particle passage from the reticulo-rumen. Fifteen percent of the water that enters the omasum is absorbed here (Krehbiel, 2014). The following abomasum is like the non-ruminant stomach, containing a glandular gastric mucosa with specialized secretory cells that produce mucus, pepsinogen, and hydrochloric acid.

For decades, experiments have been conducted to compare ruminant species’ intake and digestive capacity by feeding a wide range of diets with different ingredients and chemical compositions. In a meta-analysis, Riaz et al. (2014) stated that numerous studies have compared feed intake and nutrient digestibility between sheep and goats and between sheep and cattle. Fewer studies have made comparisons between cattle and buffaloes, and even fewer studies have been published on the comparison of feed intake and digestibility among more than two ruminant species. These comparative studies indicate that there are differences between ruminant species regarding both intake and digestive capacity at the reticulo-ruminal and total digestive tract level and that additional differences between genotypes, e.g., between Jersey versus Holstein-Friesian cows, must be considered (Beecher et al., 2014).

However, others found no systematic differences between sheep and cattle in digesting maize silage and grassland products (Aerts et al., 1984). Moreover, interspecies differences in many studies were in part, related to differences in the passage rates of solids (Bartocci et al., 1997) or fluid (Colucci et al., 1990) or both (Francoise Domingue et al., 1991; Popp et al., 1980) from the reticulo-rumen. It may therefore be argued that reticulo-ruminal in vitro systems, where reticulo-ruminal retention times or the reciprocals, i.e., outflow rates, are constantly controlled regardless of ruminant species, are less prone to be affected by species differences than in vivo studies. Therefore, the following considerations will focus on studies that have compared ruminal contents from different species in in vitro experiments.

More than 30 years ago, Kudo et al. (1984) showed that the toxic amino acid mimosine was degraded to a similar extent in vitro in ruminal fluid from both cattle and sheep and that the degradation occurred faster when the donor animals were fed a mixed forage-concentrate diet compared to forage alone. When grass silage or maize silage were incubated in a rumen simulation technique (RUSITEC) system using ruminal contents from either cows or sheep fed on three different diets, Boguhn et al. (2013) observed that in vitro nutrient degradation and microbial crude protein synthesis were more affected by the diet of the donor animals than by the animal species and was probably mediated by an adjusted microbial community. This was confirmed by Witzig et al. (2015), who analyzed the composition of the microbial community in the
### Tab. 1: Pros and cons of the reviewed *in vitro*, *ex vivo* and *in silico* methods proposed for investigating the transfer of undesirable substances from feed to food

| Technique                      | Simulated or replaced organ or tissue | Pros                                                                 | Cons                                                                 |
|--------------------------------|--------------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| Continuous culture models      | Rumen, colon, caecum                 | − No killing of animals needed.                                       | − Donor animals needed; one-time surgery required.                   |
|                                |                                      | − Long term incubation allows investigation of bacterial adaptation to xenobiotics. | − Cannulated animals need extra care.                                |
|                                |                                      |                                                                      | − Loss of ruminal protozoa                                           |
|                                |                                      |                                                                      | − No epithelial absorption and secretion                             |
|                                |                                      |                                                                      | − Time intensive                                                     |
|                                |                                      |                                                                      | − Apparatus is commonly not commercially available.                 |
| Batch culture                  | Rumen, colon, caecum                 | − No killing of animals needed.                                       | − Donor animals needed; one-time surgery required.                   |
|                                |                                      | − Easy and fast                                                      | − Cannulated animals need extra care.                                |
|                                |                                      | − Allows simultaneous investigation of several substances or dosages. | − No epithelial absorption and secretion                             |
|                                |                                      |                                                                      | − Short term incubation likely does not allow microbial adaptation to incubation conditions and xenobiotics. |
| Ussing chamber                 | All gut segments                     | − Different gut segments can be tested simultaneously.                | − Animals must be killed.                                            |
|                                |                                      |                                                                      | − Viability of tissues is limited.                                   |
|                                |                                      |                                                                      | − Costly and laborious                                              |
| Immortal epithelial cell lines | Rumen, small intestine               | − No killing of animals needed.                                       | − Sometimes different physiological behavior compared to *in vivo* conditions. |
|                                |                                      | − Rapid screening of various contaminants, their concentration and interaction | − No limited interaction with other cell types (e.g., immune cells) |
|                                |                                      | − Allows studying mechanisms at cellular level.                       | − Missing mucus layer                                               |
| Liver S9 mix and microsomes    | Liver, intestine, kidney, lung, skin  | − Reduced number of animals as organ donors needed.                  | − Results are valid on cell level but cannot necessarily be extrapolated to complex *in vivo* conditions. |
|                                |                                      | − High ethical acceptance                                            |                                                                      |
|                                |                                      | − Cheap and easy to produce.                                         |                                                                      |
|                                |                                      | − S9 contains both membrane-bound and cytosolic liver enzymes.        |                                                                      |
|                                |                                      | − Phase I and phase II reactions can be specifically investigated with the addition of appropriate cofactors. |                                                                      |
|                                |                                      | − Available from all relevant metabolizing organs.                   |                                                                      |
| Organ perfusion models         | Liver, kidney, udder                 | − Complex organ cellular interaction                                 | − Animals must be killed.                                            |
|                                |                                      | − Active *in vivo*-like transport and metabolism conditions         | − Viability of tissues is limited.                                   |
|                                |                                      | − Better understanding of quantitative contribution of the organ to certain processes | − Ischemia/reperfusion-caused cellular stress/damage                 |
| Toxicokinetic modelling        | All organs and tissues               | − No killing of animals needed.                                       | − Reliance on extensive datasets                                    |
|                                |                                      | − Can generate predictions of future scenarios.                      | − Some data needs to be generated by animal experiments (for now).    |
|                                |                                      | − Extrapolation to different species, chemicals and settings is possible. |                                                                      |
|                                |                                      | − Cheaper than animal experiments                                   |                                                                      |
RUSITEC experiment of Boguhn et al. (2013) and found that the effect of donor animal species was limited to the number of archaea, which was greater for sheep than for cows. These authors concluded that the rumen microbial community that establishes in vitro is primarily affected by the donor animal’s diet. Based on their findings, the authors suggested using a standardized approach for studying the rumen microbiota. Although ruminally cannulated animals and therefore transport distances are often too long to maintain access to the laboratories for collection time, donor animal diet, fermentation substrate, and inoculum preservation method may all have an impact on the study variables.

In accord with the observations outlined above, Henderson et al. (2015) reported that differences in ruminal microbial community compositions were predominantly determined by the diet and much less by the species. Moreover, a core microbiome was found across geographical regions and ruminant species, such that similar bacteria and archaea dominated in nearly all samples, and only protozoal communities were more variable. According to the “core bacterial microbiome” at the genus or higher levels comprises Prevotella, Butyrivibrio, and Ruminococcus, as well as unclassified Lachnospiraceae, Ruminococcaceae, Bacteroidales, and Clostridiales, providing a multitude of enzymes needed for the breakdown of nutrients (Hartinger et al., 2018; Huws et al., 2018; Morais and Mizrahi, 2019).

In summary, it appears that ruminal contents from different ruminant species are equally suitable for in vitro studies on rumen metabolism, provided that feeding management of animals and handling of ruminal fluid is standardized and procedures are applied consistently, regardless of the origin of ruminal fluid.

2.2 Continuous culture models

The rumen harbors a complex anaerobic ecosystem consisting of different niches of microbes, which enable extensive degradation and fermentation of feedstuffs. Diverse microbes have been shown to either adhere to fiber particles, be associated with the liquid phase (McAllister et al., 1994; Klevenhusen et al., 2017) or be attached to the rumen epithelium (Petri et al., 2013; Wetzels et al., 2016). Accordingly, in vitro systems simulating the rumen fermentation should ideally preserve this complex microbial community, mirroring the original rumen microbiota and fermentation processes, and therefore the in vitro fermenters must be inoculated with the original rumen microbiota from donor animals.

Ruminal contents for inoculation can be harvested either from slaughterhouses or from ruminally cannulated animals. The advantages of using ruminally cannulated animals are that their medical history is known and their feeding management can be controlled and adapted to specific research questions. Cannulated animals are commonly kept in research facilities with close access to the laboratories for in vitro incubation. Short transport distances and times ensure microbial survival. In comparison, it is often difficult to find slaughterhouses near research facilities, and therefore transport distances are often too long to maintain the ruminal microbiota. Although ruminally cannulated animals can live long, healthy lives, the cannulation itself is a surgical intervention considered to be an animal experiment and thus requires sound justification.

Several continuous culture systems were developed in the 1960s (Aafjes and Nijhof, 1967), however their applicability was often limited. Three major systems are routinely used in several laboratories worldwide today: the rumen simulation technique (RUSITEC), which was established in the 1970s by Czerkawski and Breckenridge (1977, 1979a,b); a system first described by Slyter et al. (1964), in which solid feed is added directly to the fermenters and the overflow is collected; and the dual flow continuous culture system designed by Hoover et al. (1976), in which solid feed is also added directly to the fermenters but the overflow of the liquid phase and the solid phase leave the fermenters separately with different outflow rates.

The RUSITEC system typically comprises 4 to 8 fermenters, each having a volume of 700 to 1000 mL. Artificial saliva (e.g., McDougall, 1948) is infused continuously at the bottom of the vessel, and the overflow of the liquid phase and the fermentation gases are forced through an outflow by a small positive pressure in the gas space. The overflow and the produced gas can be collected in bottles and gastight bags, respectively, for quantification and analysis. Degradability of the diets is commonly determined from synthetic fiber feed bags after 48 h of incubation. Pore sizes of the feed bags vary between studies, from 50 to 200 μm.

On the first day of a RUSITEC experiment, each fermenter is filled with a mixture of rumen fluid from at least three donor animals, as proposed by Udén et al. (2012), and artificial saliva. In addition, approximately 80 g solid material of the rumen fiber mat (Czerkawski and Breckenridge, 1977) is provided in a feed bag to enable the establishment of both liquid- and particle-associated ruminal microbes. An additional feed bag contains the experimental diet to ensure nutrient delivery to the microbes. Both bags are placed into the “food container” inside of each fermenter, which is continuously moved up and down to warrant constant mixing of the fluid and the solid phase (Czerkawski and Breckenridge, 1977). Feed bags are usually incubated in the fermenters for 48 h before being replaced with fresh ones.

In routine RUSITEC experimental runs, the digestibility of different feedstuffs or diets as well as fermentation variables such as the production of SCFA and methane can be determined in the relatively short time of 10 to 15 days (e.g., Hindrichsen et al., 2004; Khiaosa-Ard et al., 2009; Terry et al., 2018); however, longer experiments of more than 20 days also have been successfully conducted (e.g., Wallace and Newbold, 1991; Soliva et al., 2004). To obtain statistically valid results, sufficient replicates need to be obtained by repeating the experimental runs, thus prolonging the full experiment to several weeks.

One limitation of the RUSITEC system is that, although bacterial populations can be largely maintained for several days and even weeks (Ziemer et al., 2000; Wetzels et al., 2018), the abundance of protozoal populations has been shown to decrease considerably after a few days (Ziemer et al., 2000; Martinez et al., 2010b; Lengowski et al., 2016). This is likely a result of the high liquid turnover rate in comparison to the generation intervals of the protozoa (Potter and Dehority, 1973).

Single and dual flow continuous culture systems as proposed by Slyter et al. (1964) and Hoover et al. (1976) on the other hand have been shown to also maintain the protozoal populations (Mi-
et et al., 1995; Martínez et al., 2010a) over several days up to weeks, in the rumen of the donor animals (Hannah et al., 1986; Mansfield et al., 2016) demonstrated alterations in the microbial population structures and their metabolic profiles in the presence of transgenic maize, while application of a glyphosate-containing herbicide in concentrations reflecting potential exposure of dairy cows or beef cattle did not exhibit significant effects on bacterial communities in RUSITEC (Riede et al., 2016). Kowalczyk et al. (2015) studied the recovery of perfluoroalkyl acids during the incubation of contaminated feed in RUSITEC. Recent studies have investigated the effects of Fusarium toxins on nutrient utilization, the turnover of deoxynivalenol and zearalenone (Seeling et al., 2006), and the responses of the rumen microbial communities to Fusarium-contaminated feed (Strobel et al., 2008; Boguhn et al., 2010) with RUSITEC. They could show that deoxynivalenol and zearalenone were only slightly metabolized by the in vitro rumen microbiota. Koch et al. (2006) demonstrated alterations in the microbial population structures and their metabolic profiles in the presence of transgenic maize, while application of a glyphosate-containing herbicide in concentrations reflecting potential exposure of dairy cows or beef cattle did not exhibit significant effects on bacterial communities in RUSITEC (Riede et al., 2016). Kowalczyk et al. (2015) studied the recovery of perfluoroalkyl acids during the incubation of contaminated feed in RUSITEC. Recent studies have investigated the effects of Fusarium toxins on nutrient utilization, the turnover of deoxynivalenol and zearalenone (Seeling et al., 2006), and the responses of the rumen microbial communities to Fusarium-contaminated feed (Strobel et al., 2008; Boguhn et al., 2010) with RUSITEC. They could show that deoxynivalenol and zearalenone were only slightly metabolized by the in vitro rumen microbiota. Koch et al. (2006) demonstrated alterations in the microbial population structures and their metabolic profiles in the presence of transgenic maize, while application of a glyphosate-containing herbicide in concentrations reflecting potential exposure of dairy cows or beef cattle did not exhibit significant effects on bacterial communities in RUSITEC (Riede et al., 2016). Kowalczyk et al. (2015) studied the recovery of perfluoroalkyl acids during the incubation of contaminated feed in RUSITEC. Recently, Birk et al. (2018) modified the RUSITEC system to allow testing of radiolabelled metabolites of azole fungicide and thus showed that the system can also be used to work with radiolabeled test compounds.

One reason for the lack of more studies might be the relatively long duration of a complete experiment to achieve a reasonable number of replicates for statistical evaluation. Other reasons might be the change in microbial populations and community structure with prolonged time of incubation and the constant dilution by buffer infusion. Accordingly, short term batch culture approaches might be easier to conduct and more appropriate to investigate microbial effects on the fate of contaminants in ruminal fluid (see Section 2.3.).

Although continuous culture approaches can maintain a steady fermentation pattern, which is very similar to the pattern observed in the rumen of the donor animals (Hannah et al., 1986; Mansfield et al., 1995; Martínez et al., 2010a) over several days up to weeks, it remains to be answered if the in vitro degradation and biotransformation of contaminants from feeds fully represent in vivo microbial processes, as the epimural microbiota, which is associated with the ruminal epithelium, is missing in vitro, and fiber-attached microbes (i.e., bacteria and fungi) and protozoa are underrepresented. Nevertheless, continuous culture approaches can provide information on whether certain contaminants are likely to be transformed by ruminal microbes or not.

Besides the epimural microbiota, it should be stressed that so far none of the developed incubation systems include a simulation of the ruminal epithelium with its capacity for absorption of fermentation acids and possible harmful substances as well as its release of signal molecules into the rumen. The in vitro fermentation systems do not include a provision for removal of soluble substances except through the overflow. Accordingly, additional methods to investigate epithelial absorption processes are needed as outlined in Section 3.

2.3 Batch culture techniques

In vitro batch culture systems based on ruminal fluid have traditionally been used to estimate (total-tract) organic matter digestibility, and standardization and routine application of these methods was discussed already more than 50 years ago (e.g., Alexander and McGowan, 1966). Of all in vitro batch culture systems, gas production techniques have attracted the attention of researchers to study not only ruminal feed digestibility, but direct or indirect impacts of animal production on the environment (Krishnamoorthy et al., 2005). This widespread attraction became notably evident in a comprehensive special issue published in Animal Feed Science and Technology in 2005, comprising 40 publications (Krishnamoorthy et al., 2005). Topics covered in reviews and research papers, as outlined in the preface to the special issue by Krishnamoorthy et al. (2005), comprised methodologies, repeatability, application to feed nutritive evaluation and feed secondary compounds, application to investigate environmental impacts such as methane production, application to simple-stomached animals and humans, describing and predicting gas production, and quantitative possibilities for gas data. Application of a range of in vitro batch culture systems, including gas production techniques, to nutritive evaluation of food in the hindgut of humans and other simple-stomached animals was also reviewed at that time (Coles et al., 2005), and it was pointed out that, compared with techniques simulating ruminal events, in vitro digestion methods of relevance to human food evaluation lack standardization as well as in vivo validation and justification.

Generally, in vitro gas production techniques are versatile tools to study ruminal digestion and microbial metabolism, although they also have limitations (Rymer et al., 2005). More recently, Yañéz-Ruiz et al. (2016) reviewed the applicability of in vitro batch culture experiments to assess enteric methane mitigation in ruminants and provided a range of technical recommendations to harmonize techniques for feed evaluation and assessment of rumen function and methane production. The wide applicability of in vitro gas production techniques to study ruminal events is also reflected in modifications of the method, where ruminal fluid is
replaced with buffered fecal suspensions from ruminants (Aiple et al., 1992) and other herbivores (e.g., horses; Can et al., 2009), which would allow replacing ruminally fistulated animals with intact animals.

Numerous batch culture studies have been conducted to screen the effects of bioactive substances (e.g., plant secondary metabolites) on microbial activity (e.g., methane formation; reviewed by Lewis et al., 2013; Morgavi et al., 2010; Yáñez-Ruiz et al., 2016) and fermentation kinetics. However, like with the continuous culture approach, only few studies have been carried out to determine the effects of undesirable substances or contaminants, and those few studies focused mostly on fungal metabolites. For example, Asiegbu et al. (1995) and Mojtabahi et al. (2013) investigated the effects of aflatoxins, and Jeong et al. (2010) studied the effects of deoxynivalenol on in vitro gas and SCFA production. Morgavi et al. (2013) explored the methane inhibiting effects of fungal secondary metabolites from Monascus spp. using a batch culture approach, and Akkaya and Bal (2012) determined the aflatoxin binding capacities of a Saccharomyces cerevisiae extract and mycotoxin adsorbents based on hydrated sodium calcium aluminoisilicate. Using batch culture, Hahn et al. (2015) tested whether 20 commercially available products could detoxify deoxynivalenol and zearalenone.

Even fewer studies investigated whether the rumen microbial activity degrades or biotransforms a substance of interest. Caloni et al. (2000) demonstrated a low depletion rate of Fumonisin B$_1$ in batch culture, and Mobashar et al. (2012) quantified the microbial degradation of ochratoxin A using the Hohenheim gas test, distinguishing the degradation efficiency of individual microbial groups by applying antibiotics and fungicides. Likewise, by using a centrifugation protocol, Westlake et al. (1989) and Kiessling et al. (1984) demonstrated the degradation of several mycotoxins by bacterial and protozoal preparations from ovine ruminal fluid.

However, although a quick degradation of toxic compounds can sometimes be measured in batch culture, this does not always fit the observed fermentation pattern. For example, although patulin was shown to be unstable in rumen contents, decreasing by 50% after 4 h of incubation and being hardly detectable after 18 h, it was still highly toxic to in vitro rumen fermentation (Morgavi et al., 2003). The same group also found that gliotoxin, a mycotoxin often found in conserved forages, was unstable in the rumen environment, decreasing by 90% after 6 h of incubation (Morgavi et al., 2004). Others, though, demonstrated the stability of some mycotoxins in the in vitro rumen. As such, recoveries of mycophenolic acid and roquefortine C, both mycotoxins of Penicillium section roqueforti, were 79% and 41%, respectively, after 48 h of incubation (Gallo et al., 2015). Likewise, aflatoxin B$_1$ and deoxynivalenol were not degraded by ruminal microbes in vitro (Kiessling et al., 1984). Apparently, these compounds are quite resistant to microbial degradation or ruminal biotransformation and likely reach the lower gut.

Only few studies have investigated contaminants other than fungal metabolites in batch culture. For example, Majak and Cheng (1987) determined the rates of glycoside hydrolysis and hydrogen cyanide release of three different cyanogenic glyco-

3 Models for the intestinal transfer of nutrients and contaminants

The different segments of the gastrointestinal tract differ substantially within and between species regarding their anatomy and physiological function, but the epithelium lining the intestinal tract represents the first and major barrier for both nutrients and contaminants throughout.

The Ussing chamber technique is the only experimental approach for studying transepithelial transport processes that allows quantification of unidirectional flux rates of molecules across the epithelial barrier as well as the electrophysiological characterization of transport processes. It also allows studying xenobiotic metabolism by intestinal epithelial cells through targeted or non-targeted analysis of xenobiotic metabolites. This technique originally was introduced by the Danish physiologist Hans Ussing to measure ion transport processes across frog skin (Ussing, 1949; Ussing and Zerahn, 1951).

Intestinal segments are taken immediately after slaughter, rinsed with physiological saline at 4°C and opened longitudinally. The tunica mucosa is stripped of the muscular and serosal layer, and the mucosal tissue is then mounted between the two halves of an Ussing chamber with an exposed surface of up to 2 cm$^2$, thus forming a mucosal and a serosal compartment. Each side of the chamber is connected to a buffer reservoir, which is continuously gassed with carbogen (95% O$_2$, 5% CO$_2$). An isotonic buffer solution with a pH of 7.4 commonly is used on each side to mimic physiological conditions. To maintain the viability of the epithelial tissues, glucose is added at a concentration of...
10 mmol/L to the serosal buffer solution used for incubating tissues from the small intestines. For hindgut tissues, the mucosal buffer solution should contain acetate, propionate and butyrate at physiological molar proportions with an overall concentration of 60 mmol/L.

The Ussing chamber is connected to a computer-controlled voltage clamp unit. Electrodes located close to the tissues continuously measure the transepithelial potential difference (PD). Under open circuit conditions, defined currents, which induce a short-term change in PD, are applied to the tissue at regular intervals. The transepithelial tissue resistance can be calculated using Ohm’s law. Active transepithelial electrogenic transport processes generate an electric current that can be set to zero by introducing a respective short circuit current (Isc) by a further pair of electrodes. Under these conditions, the Isc is a measure for all electrogenic transport processes. When both chemical and electrical gradients are eliminated, the transport properties can be determined by measuring unidirectional flux rates from the mucosal to the serosal (Ibm) and from the serosal to the mucosal (Ibs) side of the tissue using radioactively labelled substrates. In the absence of any electrochemical gradient, the unidirectional flux rates differ significantly and result in a significant net flux (I net) when active processes are involved in either absorption or secretion.

Numerous studies have been carried out in recent years to identify the transport properties of intestinal phosphate (reviewed by Muscher-Banse and Breves, 2019) and gastrointestinal calcium in ruminants (Wilken et al., 2012; Schröder et al., 2015). The effect of SCFA on electrophysiological and co-transport properties of calcium, sodium or urea across the bovine and ovine rumen epithelium have been studied, respectively (Sehested et al., 1995; Uppal et al., 2003; Abdoun et al., 2010). The Ussing chamber technique has also been used to understand chloride secretion and intraepithelial metabolism of histamine in both the porcine colon and the bovine rumen (Aschenbach and Gäbel, 2000; Ahrens et al., 2003; Kröger et al., 2013, 2015). Whereas the flux rates of electrolytes can be quantified based on radioactively labelled substrates, the exact measurement of organic nutrients or contaminants necessitates a more laborious approach, as most organic compounds are subjected to intraepithelial metabolism. Thus, sensitive analytical methods are needed to quantify the mucosal uptake and serosal release, also of metabolites formed in the epithelium, and the potential tissue accumulation as a function of time. This approach was successfully introduced for measuring transport processes of SCFA in the porcine hindgut (Herrmann et al., 2011). In addition, epithelial transfer of the colon carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) was evaluated across the rat intestine (Nicken et al., 2013). Finally, the Ussing chamber technique has been successfully applied to measure drug transfer in human intestinal tissues (Rozehnal et al., 2012; Jöberg et al., 2013). Alternatively, the specific radioactivity in the mucosal and serosal compartments can be measured.

The gastrointestinal segments can only be incubated for limited times. Whereas rumen epithelial tissues can be incubated for at least 6-7 h, tissues from the small intestine can only be used for approximately 2.5-3.5 h; hindgut tissues survive for intermediate times. A viability test must be performed after each experiment using secretagogues such as forskolin, theophylline or carbachol or nutrients that are transported by electrogenic mechanisms to confirm tissue functionality during the experiment.

Other in vitro incubation techniques such as the everted sac technique (Harmeyer et al., 1973) can be used for absorption studies, however they do not allow the continuous control of tissue functionality like the Ussing chamber technique.

Gut epithelial cell culture models might help to understand local metabolism of xenobiotics in gastrointestinal cells, but although epithelial cell cultures from ruminal epithelium have been successfully established (e.g., Stumpff et al., 2009, 2011; Kent-Dennis et al., 2020), they have very rarely been used to analyze xenobiotic metabolism or transport. Most of the established ruminal epithelial cell culture models are based on primary ruminal cells harvested from freshly killed animals. Thus, the use of immortal cell lines to test drug metabolism and cytotoxicity (Allen et al., 2005) could reduce the number of required animals. Immortal enterocyte cell lines have been established from several species. For example, immortalized bovine epithelial cell lines from the rumen and small intestine have been used to study transport mechanisms of pathogens and to evaluate drug delivery via membranous epithelial cells (Miyazawa et al., 2010; Zhan et al., 2019). Another study established a sheep ruminal cell line to screen for possible interactions of transgenic feed and food compounds with the cells lining the rumen (Bondzio et al., 2008).

4 In vitro models for hepatic metabolism

Once nutrients or contaminants have crossed the intestinal epithelium, either via active transport, passive transcellular diffusion or the paracellular route, they are transported via blood to the liver or, when first transported with the lymph, enter the systemic blood circulation through the thoracic duct. Therefore, these substances may be subjected to different biotransformation and excretion pathways or may accumulate in different organs, including tissues used for human consumption. Understanding the fate of contaminants after intestinal absorption is thus pivotal for the assessment of risks for both animal health and consumer safety.

The liver, considered the most important metabolizing organ, consists of different cell types including hepatocytes, endothelial cells, stellate cells, Kupffer cells, pit cells, and bile duct cells. Hepatocytes account for approximately 80% of the healthy liver mass (Gerlach et al., 1994). The transporter-mediated uptake of endogenous substances and xenobiotics from the portal blood plasma into the hepatocytes occurs mainly via their sinusoidal (basolateral) membrane. In the context of metabolism, this process is referred to as phase 0 (Döring and Petzinger, 2014). In humans, transport proteins from the class of organic anion transporting polypeptides (OATP), organic anion transporters (OAT), cation transporters (OCT), and, to a lesser extent, the Na’/taurocholrate cotransporting polypeptide (NTCP) are involved in the active uptake of compounds (Müller and Jansen 1997; Marin, 2012). In contrast to the state of knowledge in humans and exper-
mentals, little is known about the occurrence of uptake transporters in ruminant tissues. It was shown that OATPs can also be found in cattle tissues (Liu et al., 2013; Xiao et al., 2014).

After uptake, hepatocytes are responsible for converting lipophilic (toxic) xenobiotics such as dietary and environmental pollutants (plant and fungal toxins, pesticides, herbicides, etc.) and drugs into water-soluble forms, so-called metabolites, which can be eliminated from the body via the excretory organs, i.e., kidney and intestine. However, this process can sometimes activate previously non-toxic substances into toxic metabolites or increase the potency of already toxic substances. Accordingly, the liver not only has a high detoxification capacity, but also an activating potential of xenobiotics (e.g., pyrrolizidine alkaloids; Mattocks, 1986; Roeder, 2000; Wiedenfeld and Edgar, 2011; or aflatoxins; Kulman et al., 2000; Alvarado et al., 2017).

The two phases of biotransformation of both endogenous and exogenous compounds are referred to as phase I and phase II reactions. Phase I reactions are catalyzed by enzymes such as cytochromes P-450 (CYPs) or other oxidoreductases. Often, hydroxyl groups are introduced into the molecules, e.g., hydroxyl phospholipids. Further phase I reactions are hydration and dehydrations. Phase II enzymes such as uridine diphosphate glucurono-syltransferases (UGTs), glutathione S-transferases (GSTs), sulfotransferases (SULTs) or amino acid transferases conjugate nucelophilic groups of xenobiotics or their oxidized phase I metabolites with highly ionized hydrophilic molecules such as glucuronic acid (He et al., 2010; Maul et al., 2012; Li et al., 2017a), glutathione (Larsson et al., 1994; Muluneh et al., 2018), sulfamate (Smith and Shelver, 2002; Li et al., 2017a) or amino acids (Knights et al., 2007). For this purpose, phase II enzymes require cofactors. Conjugates can be further metabolized or passed through an enterohepatic cycle. Excretion of phase I metabolites or conjugates can occur renally, biliarily, by sweat or by breath.

Species differences in metabolism between humans and animal species are of great importance for the risk assessment of xenobiotics. If the metabolism of compounds in humans and animals varies qualitatively or quantitatively, this leads to different consequences that enter the animal after being absorbed from the gut. For this, the substance of interest is incubated with a buffer containing the microsomes and cofactors (Jia and Liu, 2007; Knights et al., 2016). The activity of the CYPs and UGTs, however, can vary depending on the buffer. In general, 50-100 mM Tris HCl (pH 7.5 at 37°C) or potassium or sodium phosphate buffer (pH 7.4) is suitable, but UGTs appear more active in Tris buffers than in phosphate buffers (Boose and Miners, 2002; Engtrakul et al., 2005; Argikar et al., 2016; Badée et al., 2019). Metabolism studies require the addition of cofactors for the target enzyme, e.g., 1-4 mM NADPH for CYPs or 2 mM uridine diphosphate glucuronic acid (UDPGA) for UGTs. The protein concentration of the enzyme solution can be adjusted depending on the application and susceptibility of the substrate (Jones and Houston, 2004). Metabolization can be started by preheating the substrate, buffer and cofactors at 37°C and adding the cold enzyme fraction. An alternative approach is to preheat the enzymes, substrate and buffer and start metabolization by adding the cofactor. The reaction is stopped at specified times by adding ice-cold organic solvent (e.g., methanol, acetoniirile optionally acidified with formic acid or ammonium formate). The samples should then be well mixed by vortex and stored for at least 20 min at -20°C to -80°C to facilitate precipitation of proteins and salts, which can finally be sedimented by centrifugation (Jia and Liu, 2007; Knights et al., 2016). The enzymatic degradation of the substrate and the identification of metabolites may be investigated using, e.g., liquid chromatography coupled to mass spectrometry, nuclear magnetic resonance spectroscopy, UV or radioactivity detection (Wen and Zhu, 2015; De Vijlder et al., 2018; Foroutan et al., 2019; Zhang et al., 2019; Thiel et al., 2019; Tolonen and Pelkonen, 2015).

In vitro hepatic metabolism studies have been conducted for numerous contaminants (e.g., Maul et al., 2012; Kolrep et al., Yoshioka et al., 2016), and subcellular fractions derived from liver homogenates. CYP, UGT or SULT isoenzymes can be expressed recombinantly in insect cells (supersomes) or E. coli (bactosomes) (Asseff et al., 1989; Kost et al., 2005).

Subcellular fractions of liver tissue can be divided by differential centrifugation into the 9000 × g supernatant fraction (S9 fraction), cytosol and microsomes (von Jagow et al., 1965; Hubbard et al., 1985; Graham, 2002; Richardson et al., 2016). The S9 fraction, which is also commercially available from a variety of species, contains both the enzymes present in the cytosol (e.g., SULTs, GSTs) and those bound in the microsomes (e.g., CYPs, UGTs). After cell lysis, a solution containing broken cells, small fragments of the plasma membrane and the endoplasmic reticulum is obtained, while the organelles (e.g., mitochondria, nuclei, lysosomes) remain intact.

This lysate can then be subjected to a series of centrifugation steps in an ultracentrifuge to be fractionated into several components, e.g., into microsomes and cytosol (1 h at 104'000 × g). The microsomal fraction consists of vesicles that are formed from the endoplasmic reticulum, containing phase I and phase II enzymes (Kedderis, 2018; Sanchez and Kauffman, 2010). Microsomes are widely used to evaluate the metabolic stability and metabolite formation of a compound. Accordingly, they can be used to identify possible metabolization of undesirable substances that enter the animal after being absorbed from the gut. For this, the substance of interest is incubated with a buffer containing the microsomes and cofactors (Jia and Liu, 2007; Knights et al., 2016). The activity of the CYPs and UGTs, however, can vary depending on the buffer. In general, 50-100 mM Tris HCl (pH 7.5 at 37°C) or potassium or sodium phosphate buffer (pH 7.4) is suitable, but UGTs appear more active in Tris buffers than in phosphate buffers (Boose and Miners, 2002; Engtrakul et al., 2005; Argikar et al., 2016; Badée et al., 2019). Metabolism studies require the addition of cofactors for the target enzyme, e.g., 1-4 mM NADPH for CYPs or 2 mM uridine diphosphate glucuronic acid (UDPGA) for UGTs. The protein concentration of the enzyme solution can be adjusted depending on the application and susceptibility of the substrate (Jones and Houston, 2004). Metabolization can be started by preheating the substrate, buffer and cofactors at 37°C and adding the cold enzyme fraction. An alternative approach is to preheat the enzymes, substrate and buffer and start metabolization by adding the cofactor. The reaction is stopped at specified times by adding ice-cold organic solvent (e.g., methanol, acetoniirile optionally acidified with formic acid or ammonium formate). The samples should then be well mixed by vortex and stored for at least 20 min at -20°C to -80°C to facilitate precipitation of proteins and salts, which can finally be sedimented by centrifugation (Jia and Liu, 2007; Knights et al., 2016). The enzymatic degradation of the substrate and the identification of metabolites may be investigated using, e.g., liquid chromatography coupled to mass spectrometry, nuclear magnetic resonance spectroscopy, UV or radioactivity detection (Wen and Zhu, 2015; De Vijlder et al., 2018; Foroutan et al., 2019; Zhang et al., 2019; Thiel et al., 2019; Tolonen and Pelkonen, 2015).
Hayes et al. (1977) determined more than five phase I metabolites after incubation of aflatoxin B1 with bovine liver microsomes. In a subsequent feeding study, one of these metabolites, aflatoxin M1, was extracted using organic solvents from kidney, liver and mammary gland obtained from a cow receiving 7.31 mg/day aflatoxin B1 over a 14-day period.

The metabolism of various toxic pyrrolizidine alkaloids present in many plants belonging to the families of Asteraceae has also been studied using liver tissue fractions from cattle (Huan et al., 1998; Düringer et al., 2004; He et al., 2010; Fashe et al., 2015; Kolrep et al., 2018; Muluneh et al., 2018). In vivo studies with cows provide evidence that some of these pyrrolizidine alkaloids or their metabolites are transferred to milk (Dickinson et al., 1976; Johnson, 1976; Candrian et al., 1991; Hoogenboom et al., 2011).

When the in vitro liver metabolism is known and the metabolites can be identified, it can be judged whether there is a need to measure these metabolites in food of animal origin (e.g., milk, meat). However, due to the often-lacking standards for the resulting metabolites, their discovery remains a challenging task.

In phase III of metabolism, the metabolites as well as unmetabolized substrates are transported out of the hepatocytes by a transporter-mediated efflux either via the basolateral membrane into the portal blood for renal excretion or via the canalicular (apical) membrane into the bile for fecal excretion (Döring and Petzinger, 2014; Müller and Jansen 1997; Marin, 2012). These transport proteins belong to the superfamily of ATP-binding cassette (ABC) transporters and are well characterized in humans.

The data on the occurrence of efflux transport proteins in farm animals is as limited as on uptake transport proteins. Results exist on the expression of efflux transporter ABCG2/BCRP in tissue barriers of lactating dairy cows, sheep and goats (Lindner et al., 2013). The large data gaps regarding the transport mechanisms of xenobiotics in farm animal tissues have been pointed out by several scientists (Martínez et al., 2018; Virkel et al., 2019; Rosa, 2020). There remains an urgent need for research on this topic.

5 Ex vivo organ perfusion models

Biotransformation processes are the result of a complex interplay of different metabolic and cellular systems that cannot be captured with simple cell culture models. Excretion pathways via bile, urine or milk, putative re-absorption from the intestinal tract or further metabolism after initial hepatic biotransformation contribute to the complexity. Thus, ex vivo perfusion models including whole organs or even body parts may help to generate information about the fate of contaminants in the body without using live animals (Daniel et al., 2018). Organs may often be obtained from slaughterhouses instead of from purpose-killed animals (Grosse-Siestrup et al., 2002). Such perfusion models have a long tradition in toxicological testing and for studying and improving the preservation conditions for organs intended for transplantation in humans. They may also be used to gain insight into the metabolism and transfer of contaminants.

5.1 Kidney and liver perfusion models

The isolated and perfused liver already was described for various animal species almost a century ago (Plattner, 1924; Höber and Titajew, 1930; Haywood et al., 1945; Gunberg et al., 1955). The first model of a perfused bovine (calf) liver was described in the 1960s (Chapman et al., 1961). Studies using isolated perfused porcine and dog kidneys were conducted even earlier to study factors influencing urine formation (Locbell, 1849; Starling et al., 1925; Nizet, 1975). Today, studies are performed with porcine livers and kidneys from slaughtered animals due to their anatomical similarity to humans, their model role for circulatory death, and their accessibility (Grosse-Siestrup et al., 2003; Dondossola et al., 2019). The isolated porcine or bovine liver or kidney can be perfused with modified artificial fluids or heparinized blood and allows the study of the biotransformation of molecules of interest (e.g., contaminants), excretion through the venous blood, urine or bile, and possible adverse effects on hepatic or renal function. Sampling of arterial and venous fluids as well as bile allows toxicokinetic modelling at whole-organ level.

5.2 The perfused bovine udder

A variable amount of systemically available contaminants is excreted with the milk. This depends on their lipophilicity and degree of ionization, because the pH of milk (pH 6.5 to 6.7) is lower than that of blood. Therefore, partitioning of weak acids through the blood milk barrier is limited. Accordingly, the ratio of the ionized and the non-ionized form of compounds is high in the blood plasma.

The penetration of the blood-milk barrier by a compound can be predicted in silico based on the pH, pK-dependent partitioning phenomenon. Ziv and Rasmussen (1975), Shen-Tov et al. (1997) and others calculated the transfer rate of various compounds from blood plasma into milk and found a good correlation of the predicted data with results of in vivo studies (concentration in milk samples of treated cows). Possible inducible active transport processes should also be considered (Halwachs et al., 2013; Mahnke et al., 2016).

First perfusion models of the bovine udder already were described in the 1950s (Peeters and Massart, 1952; James et al., 1956; Verbeke et al., 1957). Since the isolated perfused bovine udder first was used to study the distribution of antibiotics in the udder tissue (Kietzmann et al., 1993), various ex vivo studies were performed to study the tissue distribution of β-lactam antibiotics, marbofloxacin and cefquinom (Ehinger and Kietzmann, 1998, 2000a,b, 2001, 2006; Kietzmann et al., 2008). Advantages of using the isolated perfused udder are that both tissue and milk samples can be taken repeatedly at various time points. A disadvantage is the relatively short duration of tissue viability of up to about 8 h, which must be controlled during perfusion. Suitable viability parameters that can be measured in the perfusate include glucose consumption, lactate production, and lactic dehydrogenase (LDH) concentration. Additionally, cell viability can be determined in tissue samples by cell viability tests. Another disadvantage is that isolated organs are deprived of nervous regulation and lymph drainage.
Medium sized udders of slaughtered healthy lactating cows are used. Directly after slaughtering, blood clots in the gland’s vessels are cleared using heparinized Tyrode’s solution. In the laboratory, the udder can be fixed in a “natural” position using a metal frame. The perfusion must be started within minutes after insertion of silicone tubes into the large arteries of each udder half. The large veins are also cannulated to allow sampling and removal of the perfusate. Smaller veins are closed using artery forceps. After an equilibration period, the perfusion is continued with a fluid containing the test compound. In most experiments by Ehinger and Kietzmann (1998, 2000a,b, 2001, 2006) and Kietzmann et al. (2008), the isolated bovine udder was perfused with Tyrode’s solution, which resembles lactated Ringer’s solution but contains magnesium, a sugar (usually glucose) as an energy source, and bicarbonate and phosphate instead of lactate. Perfusion with heparinized and diluted blood also is possible. However, selecting the most suitable perfusion medium depends on the solubility of the test compound in the perfusion fluid. When the time-dependent blood plasma concentration of a test compound or its metabolites is known, the udder perfusion can be performed with its adapted concentrations to simulate realistic situations. The measured tissue and milk concentrations allow calculating the amount of test compound that is eliminated via milk.

Unlike in vivo experiments on intact animals, the isolated perfused bovine udder model enables the investigator to retain control over various internal and external variables, such as type and composition of the perfusate or a certain treatment before or during the experiment, e.g., the impairment or improvement of organ physiology.

6 Integrating in vitro and in silico generated data using kinetic modelling

In vitro, ex vivo and in silico methods produce data about individual in vivo physiological processes describing the transfer of undesirable substances in ruminants. To integrate these data from individual methods into a coherent whole, a systems biology approach like kinetic modelling is required.

6.1 Toxicokinetic modelling

Kinetic modelling is a mathematical tool used to predict absorption, distribution, metabolism and excretion (ADME) of a substance of interest in live organisms. The substance may be a drug, in which case the term used is pharmacokinetics (PK), or a toxin or toxicant, in which case the term used is toxicokinetics (TK). Kinetic models describe the fate of a substance entering an organism by compartmentalizing the organism and using differential equations based on biochemical principles to describe the mass flows between various compartments and the chemical reactions happening within. These compartments may be ad-hoc groups of tissues and organs (Numata et al., 2014) or represent well-defined physiological structures, e.g., the extracellular space of the liver (Savvateeva et al., 2020). In the former case, the models are called compartment TK models, and in the latter case they are called physiologically-based toxicokinetic models (PBTK). Techniques exist to reduce the complexity incurred by including many physiological tissues and combine them into simpler compartment models, bridging TK and PBTK models (Pilari and Huisinga, 2010). Both flows and reactions in the compartments are represented with corresponding parametric differential equations, which yield the change in amount or concentration in each compartment over time.

Interest in PBTK models is growing, as their ability to accurately reflect the physiology of the underlying modelled processes makes them more accurate provided sufficient data is available. Modeling physiological processes may increase the predictive ability of PBTK models compared to empirical TK models. There is much potential in this field, and research is progressing fast, especially in the field of animal health (Lin et al., 2016). PBPK/TK models have been developed for ruminants (goats, cows) by Leavens et al. (2012) and Li et al. (2018). While the goat model by Leavens et al. (2012) was developed for veterinary purposes, the cow model was developed to ensure compliance with maximum residue levels of penicillin in milk.

In vitro-to-in vivo extrapolation (IVIVE) in conjunction with PBPK/TK is an approach to bridge in vitro and in vivo data and to examine the key mechanisms determining the kinetics. PBTK modeling has also been used to address IVIVE for animal-free risk assessment (Fabian et al., 2019). In that study, an eight compartment PBTK rat model was developed and its predictive efficacy assessed by comparing it to relevant in vivo studies.

Kinetic models can be built using available knowledge about physical and computational chemistry, animal physiology (models of systems), animal experimental data (empirical models) or a combination thereof (hybrid models) (Bonate, 2011). To describe the fate of a substance in an organism with (PB)TK modelling, the optimal kinetic equation parameters must be estimated, i.e., the parameters that ensure the model equations follow a time trajectory consistent with what one would observe in vivo. Data from in vivo feeding experiments can be used and fitted to the model equations to identify the kinetic parameters of interest (Bonate, 2011). Alternatively, one can use data derived from the literature, i.e., from in vitro models and from in silico prediction algorithms (computational toxicology) (Bolt and Hengstler, 2020) to obtain estimates for TK parameters, reducing or eliminating altogether the need for animal experiments (Paimi et al., 2019). This approach poses challenges but at the same time has much potential (Lin and Wong, 2017), both for the sake of animal and human health and for saving time and money in the risk assessment of new undesirable substances. In the following, we present a summary of how (PB)TK models can be used as the final step to validate, complete and integrate the information gained from in vitro models of ruminants, such as the ones presented in the previous sections.

6.2 Integrating in vitro and ex vivo data using kinetic modeling

PBTK models are powerful tools that can be used to simulate the change in concentration of a xenobiotic in tissues (local internal exposure) of interest upon (external) exposure. They can be
cause of the heterogeneity of gut microbiota, which may metab-
olize the modelled substance. The digesta flow can be estimat-
ed from physiological equations and animal science literature.
Absorption into the blood can be estimated with data from Uss-
ing chamber experiments. Predicting the chemical reactions that
a substance may undergo requires a different approach. As dis-
cussed above, continuous or batch culture models can be used to
investigate the metabolization of contaminants by ruminal mi-
icrobiota. If reaction rates for metabolites can be obtained, they
can be included in the model, improving its performance and ac-
curacy.

Intestine
Two key parameters for the intestinal compartment are the ab-
sorption rate into blood and the rate of fecal excretion. The ab-
sorption rate from the intestine depends on a variety of factors,
including the membrane’s permeability, the substance’s stability
in the digesta, and the time available for absorption (Cho et al.,
2014). The stability can be assessed by using incubation meth-
extrapolated across species, doses, routes of administration and
compounds, as well as used for IVIVE (Li et al., 2017b; Fabian et
al., 2019; Lin et al., 2020). An example of how PBTK can be em-
ployed to extrapolate the fate of xenobiotics from dairy cattle to
other species can be found in MacLachlan (2009). Such models
require detailed knowledge on chemical attributes (e.g., partition
coefficients) and, in the case of PBTK, of physiological variables
(e.g., cardiac output, organ weights, and blood flow rates) (Lin et
al., 2020), some of which can be challenging to obtain without in
vivo data. In the next paragraphs, the feasibility of determining
such essential variables using in vitro, ex vivo and literature data
will be discussed for each of the organs and tissues most relevant
for assessing the kinetics of xenobiotics in ruminants exposed
through the oral route (Fig. 1).

Rumen
The rumen compartment is of crucial importance when model-
ling the fate of xenobiotics after oral intake, not only because it
determines the rate of digesta flow to the intestine, but also be-

Fig. 1: A representative example of a toxicokinetic model for ruminants
The colored compartments represent the various organs or tissues; the flows of contaminants in and out of compartments are represented
by kinetic parameters, which are given next to the corresponding arrow. The concentration change of a contaminant (and potentially its
metabolites) in each compartment is expressed in the form of a parametrized differential equation. One subscript letter: internal reactions
(e.g., liver metabolism, microbiome-mediated reactions, etc.). Two subscript letters: mass flow from one compartment (first letter) to
another (second letter). Three subscript letters: excretion mass flow from one compartment (first letter) followed by the “o” for output
(second letter), followed by the excretion route (last letter). f, feed; x, exposure route; r, ruminal solids; j, ruminal fluid; i, intestine; b, blood;
l, liver; m, muscle tissue; a, adipose tissue; k, kidney; u, udder; of, out feces; om, out milk; ou, out urine
ods, as mentioned above. The time available for absorption is the transit time of digesta in the intestine, which can be found in animal science literature. As with the rumen, the permeability of the intestinal walls to a substance and epithelial metabolism can be investigated with the Ussing chamber technique. Fecal excretion rates can be calculated based on physiological equations and bioenergetics (Gabel et al., 2003). If the absorption rate of a substance into the blood and the frequency and amount of fecal excretion are known, substance concentrations in both feces and blood can be estimated.

**Adipose, muscle and blood-tissue compartments**

The blood-tissue compartment connects all other compartments in the (PB)TK model, reflecting the physical reality of the animal organism. The influx of substance from the GIT, which plays a significant role in determining the substance levels in blood, has already been described. Likewise important are the flows to and from the remaining compartments. Peripheral compartments are relevant for substances that accumulate in kinetically slower tissues (such as lipophilic substances in adipose tissue or lead in bones), and muscle tissue may be relevant for substances that can bind to proteins.

An effective approach in such cases is the estimation of partition coefficients. A partition coefficient is the ratio of concentrations of a compound between two distinct phases (in this case tissues or groups of tissues) at equilibrium. To apply partition coefficients, it is unnecessary that the transfer kinetics for all processes are in equilibrium or steady state, but it is crucial that the local distribution kinetics between the two relevant phases is fast compared to other kinetic processes (quasi-equilibrated state). The distribution of a substance from the blood to those tissues can be modelled as the diffusion between two phases of a solute, a process that may be adequately described by a partition coefficient. A range of such partition coefficient prediction algorithms exists, from those that use a simple octanol-water partition coefficient to account for the transfer of lipopholic substances into the tissue (Hermens et al., 2013) to more complex formulations, where the biological tissue is represented by a complex mixture of biochemicals (Schmitt, 2008; Poulin and Theil, 2000). Several *in vivo* and *in silico* methods are available to predict partition coefficients (Graham et al., 2012). More sophisticated predictors of partition coefficients, like those based on the polyparameter linear free energy relationship (pLFER) (Endo et al., 2013), can be used to capture the complex thermodynamics of biological molecules. Such an approach was applied by Savvateeva et al. (2020) to develop (PB)TK models of contaminants in growing pigs. Nevertheless, using partition coefficient prediction methods has limitations, given that they fail to represent differences in lipid types and other specific molecular interactions and non-covalent binding relevant for biological systems.

Specific binding to blood proteins can be studied using *in vitro* methods (MacManus-Spencer et al., 2010). For instance, serum albumin is the most important blood protein carrier for perfluoroalkyl acids (PFAAs) (Forsthuber et al., 2020). This binding can be explicitly built into PBTK models, where only the free, unbound fraction is available for exchange with other tissue compartments (Loccisano et al., 2013).

**Liver**

The liver plays an essential role in the metabolism of chemicals in the body. It can act both as a sink (detoxification) or as a source (bioactivation) of xenobiotics. It is therefore important to establish whether and how a xenobiotic is processed in the liver and to determine hepatic clearance (the rate at which a substance is degraded or transformed in the liver). Theoretical models that describe hepatic clearance are available for humans (Laveé and Funk, 2007) and can be adapted to other mammals such as ruminants. Information about the blood perfusion rate to the liver can be obtained from animal science literature (e.g., Lescoat et al., 1996). The actual liver reactions can be investigated either using *ex vivo* perfused liver models or *in vitro* hepatic models as described above. The liver biochemical reactions with their rate parameters can then be included in the PBTK model, encompassing possible self-induced metabolism (Savvateeva et al., 2020). Like other tissues, the liver may contain proteins that specifically interact with and store xenobiotics.

**Kidneys**

The kidney compartment in the model predicts the fraction of substance excreted via urine. Although *no in vitro* model exists that can simulate complete kidneys, the *ex vivo* perfused kidney model or *in silico* models can be used to obtain data without animal experiments. Kidneys are a challenging organ to model, since they are key not only to excretion processes but also to osmoregulation, which is subject to complex hormonal regulation and causes kidney activity to steadily change in response to internal and external stimuli. Nevertheless, it is possible to focus on physiological parameters of central relevance: glomerular filtration, tubular reabsorption and secretion. Depending on the modelled substance, reabsorption and secretion may be excluded from the model, whereas the glomerular filtration rate (GFR) is indispensable. In fact, the GFR has been shown to be proportional to renal drug clearance in some human PBPK renal models (Janků, 1993), making it one of the most relevant parameters for modelling renal excretion of xenobiotics. Although to date no such computational model exists for ruminants, the advances in predicting human renal clearance and urinary excretion (Dodderedy et al., 2006; Huang and Isosherranen, 2018; Watanabe et al., 2019) suggest the feasibility of developing similar prediction models for ruminants.

**Udder**

The udder compartment can be modelled by knowing the blood perfusion rate and the milk production rate, available in animal science literature (e.g., NRC, 1988), as well as other kinetic parameters quantifying the transfer through the blood-udder barrier

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1. Sontag, E. D. (2011). Lecture notes on mathematical systems biology. https://www.coursehero.com/file/41362918/Mathematical-Systems-Biologypdf/
There are still many challenges to using kinetic modelling to predict the transfer of contaminants into ruminant-derived food. There are still many challenges to using kinetic modelling to predict the transfer of undesirable substances in ruminants to animal-derived food without the need for animal experiments. Although several studies could successfully generate predictive models for the transfer of xenobiotics in the animal body (Strikwold et al., 2017; Zhang et al., 2018, 2020; Cheng and Ng, 2017), a range of issues still must be addressed. The IVIVE represents a bottleneck in the development of models, and the estimation of model uncertainties may be difficult without independent datasets for evaluation and calibration. In some cases, industry-generated in vivo data, e.g., for pesticides and veterinary pharmaceuticals, can be used as validation datasets to avoid further animal experimentation. The search continues for alternatives to the comparison of model output with data from in vivo experiments as a validation approach. Another issue in the integration of in vitro generated data with in silico methods is the heterogeneity of the various methods’ data output. This increases the complexity of the task and poses problems like the propagation of systematic and random errors in measurements, which can be difficult to estimate.

Despite the current issues around relying solely on mathematical modeling to predict the transfer of contaminants in animals, there are encouraging signs, as research is advancing rapidly, and the methods continue to improve. Not having to rely on animal experiments to predict the transfer of xenobiotics could speed up the risk assessment of new chemicals while reducing costs, contributing to both human and animal welfare.

7 Conclusions

Several of the here reviewed in vitro, ex vivo and in silico methods were established decades ago and are applied in laboratories worldwide. However, the number of studies centered on the transport and metabolism of undesirable substances in the ruminant animal is still modest. Although the applicable methods can adequately simulate individual body compartments, the challenge of aligning all their outputs to follow a xenobiotic and its metabolites of interest through the animal body remains. In silico approaches using toxicokinetic modelling offer the potential to integrate the data produced by a variety of methods and perform effective in vitro to in vivo extrapolation (IVIVE). The goal is to employ such methods to produce results comparable to in vivo experiments and to simulate diverse feeding and production scenarios. This represents a viable way to close the remaining gap between in vitro and in vivo results.

References

Aafjes, J. H. and Nijhof, J. K. (1967). A simple artificial rumen giving good production of volatile fatty acids. Brit Vet J 123, 436-446.

Abdoun, K., Stumpff, F., Rabbani, I. et al. (2010). Modulation of urea transport across sheep rumen epithelium in vitro by SCFA and CO2. Am J Physiol Gastrointest Liver Physiol 298, 190-202. doi:10.1152/ajpgi.00216.2009

Aerts, J. V., De Boever, J. L., Cottyn, B. G. et al. (1984). Comparative digestibility of feedstuffs by sheep and cows. Anim Feed Sci Technol 12, 47-56. doi:10.1016/0377-8401(84)90035-X

Ahrens, F., Gabel, G., Garz, B. et al. (2003). Histamine-induced chloride secretion is mediated via H2-receptors in the pig proximal colon. Inflamm Res 52, 79-85. doi:10.1007/s000110300005

Aiple, K. P., Steingass, H. and Menke, K. H. (1992). Suitability of a buffered faecal suspension as the inoculum in the Hohenheim gas test. 1. Modification of the method and its ability in the prediction of organic matter digestibility and metabolizable energy content of ruminant feeds compared with rumen fluid as inoculum. J Anim Physiol Animal Nutri 67, 57-66. doi:10.1111/j.1439-0396.1992.tb00583.x

Akayka, M. R. and Bal, M. A. (2012). Efficacy of modified yeast extract and HSCAS containing mycotoxin adsorbent on ruminal binding characteristics of various aflatoxins. Kasfıca Vet Fak Derg 18, 951-955. doi:10.9775/kvfld.2012.6838

Alexander, R. H. and McGowan, M. (1966). The routine determination of in vitro digestibility of organic matter in forages – An investigation of the problems associated with continuous large-scale operation. Grass Forage Sci 21, 140-147. doi:10.1111/j.1365-2494.1966.tb00462.x

Allen, D. D., Caviedes, R., Cárdenas, A. M. et al. (2005). Cell lines as in vitro models for drug screening and toxicity studies. Drug Develop Industr Pharm 31, 757-768. doi:10.1080/03639040500216246

Alvarado, A. M., Zamora-Sanabria, R. and Granados-Chinchilla, F. (2017). A focus on aflatoxins in feedstuffs: Levels of contamination, prevalence, control strategies, and impacts on animal health. In L. B. Abdulra’uf (ed.), Aflatoxin – Control, Analysis, Detection and Health Risks. INTECH Open Science. doi:10.5772/intechopen.69468

Argikar, U. A., Potter, P. M., Hutzler, J. M. et al. (2016). Challenges and opportunities with non-CYP enzymes aldehyde oxidase, carboxylesterase, and UDP-glucuronosyltransferase: Focus on reaction phenotyping and prediction of human clearance. AAPS J 18, 1391-1405. doi:10.1208/s12248-016-9962-6

Aschenbach, J. R., Zebeli, Q., Patra, A. K. et al. (2019). Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. J Dairy Sci 102, 1866-1882. doi:10.3168/jds.2018-15243

Aschenbach, J. R. and Gabel, G. (2000). Effect and absorption of histamine in sheep rumen: Significance of acidic epithelial damage. J Anim Sci 78, 464-470. doi:10.2527/2000.782464x

Asiegbu, F. O., Paterson, A., Morrison, I. M. et al. (1995). Effects
of cell wall phenolics and fungal metabolites and methane and acetate production under in vitro rumen conditions. *J Gen Appl Microbiol* 41, 475-485. doi:10.2323/jgam.41.475

Asseffá, A., Smith, S. J., Nagata, K. et al. (1989). Novel exogenous heme-dependent expression of mammalian cytochrome P450 using baculovirus. *Arch Biochem Biophys* 274, 481-490. doi:10.1016/0003-9866(89)90461-X

Badée, J., Qiu, N., Parrott, N. et al. (2019). Optimization of experimental conditions of automated glucuronidation assays in human liver microsomes using a cocktail approach and ultra-high performance liquid chromatography-tandem mass spectrometry. *Drug Metab Dispos* 47, 124-134. doi:10.1124/dmd.118.084301

Bartocci, S., Amici, A., Verna, M. et al. (1997). Solid and fluid passage rate in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. *Livest Prod Sci* 52, 201-208. doi:10.1016/S0301-6226(97)00132-2

Beecher, M., Buckley, F., Waters, S. et al. (2014). Gastrointestinal tract size, total-tract digestibility, and rumen microflora in different dairy cow genotypes. *J Dairy Sci* 97, 3906-3917. doi:10.3168/jds.2013-7708

Belanche, A., Palma-Hidalgo, J. M., Neijam, I. et al. (2019). In vitro assessment of the factors that determine the activity of the rumen microbiota for further applications as inoculum. *J Sci Food Agric* 99, 163-172. doi:10.1002/jsfa.9157

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 70, 567-590. doi:10.1152/physrev.1990.70.2.567

Birk, B., Stähle, A., Meier, M. et al. (2018). Investigation of ruminal xenobiotic metabolism in a modified rumen simulation system (RUSITEC). *ALTEX* 35, 379-389. doi:10.14573/alex.1712221

Boase, S. and Miners, J. O. (2002). In vitro-in vivo correlations for drugs eliminated by glucuronidation: Investigations with the model substrate zidovudine. *Brit J Clin Pharmacol* 54, 493-503. doi:10.1046/j.1365-2125.2002.01669.x

Boguhn, J., Neumann, D., Helm, A. et al. (2010). Effects of concentration proportion in the diet with or without Fusarium toxin-contaminated triticale on ruminal fermentation and the structural diversity of rumen microbial communities in vitro. *Arch Anim Nutr* 64, 467-483. doi:10.1080/1745039X.2010.511515

Boguhn, J., Zaber, T. and Rodehutscord, M. (2013). Effect of donor animals and their diet on in vitro nutrient degradation and microbial protein synthesis using grass and corn silages. *J Anim Physiol Anim Nutr* 97, 547-557. doi:10.1111/j.1439-0396.2012.01295.x

Bolt, H. M. and Hengstler, J. G. (2020). The rapid development of computational toxicology. *Arch Toxicol* 94, 1371-1372. doi:10.1007/s00204-020-02768-5

Bonate, P. L. (2011). *Pharmacokinetic-Pharmacodynamic Modeling and Simulation*. Springer US. doi:10.1007/978-1-4419-9485-1

Bondzio, A., Stumpff, F., Schön, J. et al. (2008). Impact of Bacillus thuringiensis toxin Cry1Ab on rumen epithelial cells (REC) – A new in vitro model for safety assessment of recombinant food compounds. *Food Chem Toxicol* 46, 1976-1984. doi:10.1016/j.fct.2008.01.038

Busquet, M., Calsamiglia, S., Ferret, A. et al. (2005). Effect of garlic oil and four of its compounds on rumen microbial fermentation. *J Dairy Sci* 88, 4393-4404. doi:10.3168/jds.S0022-0302(05)73126-X

Caloni, F., Spotti, M., Auerbach, H. et al. (2000). In vitro metabolism of Fumonisin B1 by ruminal microflora. *Vet Res Commun* 24, 379-387. doi:10.1023/A:1006422200226

Can, A., Hummel, J., Mobashar, M. et al. (2009). Comparison of sheep ruminal fluid with sheep and horse faeces as inoculum for in vitro gas production measurements. *J Appl Anim Res* 35, 143-148. doi:10.1080/09712119.2009.9707004

Candrian, U., Zweifel, U., Luethy, J. et al. (1991). Transfer of orally administered [3H]-selenophyline into cow’s milk. *J Agirc Food Chem* 39, 930-933. doi:10.1021/jf00005a026

Chapman, N. D., Goldsworthy, P. D., Wolviler, W. et al. (1961). The isolated perfused bovine liver. *J Exp Med* 113, 981-995. doi:10.1084/jem.113.6.981

Cheng, W. and Ng, C. A. (2017). A permeability-limited physiologically based pharmacokinetic (PBPK) model for perfluorooctanoic acid (PFOA) in male rats. *Environ Sci Technol* 51, 9930-9939. doi:10.1021/acs.est.7b02602

Cho, H. J., Kim, J. E., Kim, D. D. et al. (2014). In vitro-in vivo extrapolation (IVIVE) for predicting human intestinal absorption and first-pass elimination of drugs: Principles and applications. *Drug Dev Ind Pharm* 40, 989-998. doi:10.3109/03639045.2013.831439

Coles, L. T., Moughan, P. J. and Darragh, A. J. (2005). In vitro digestion and fermentation methods, including gas production techniques, as applied to nutritive evaluation of foods in the hindgut of humans and other simple-stomached animals. *Anim Feed Sci Technol* 123-124, 421-444. doi:10.1016/j.anifeedsci.2005.04.021

Colucci, P. E., MacLeod, G. K., Grovum, W. L. et al. (1990). Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. *J Dairy Sci* 73, 2143-2156. doi:10.3168/jds.S0022-0302(90)78895-9

Craig, A. M., Latham, C. J., Blythe, L. L. et al. (1992). Meta-analysis of computational toxicology. *Brit J Nutr* 68, 201-208. doi:10.1079/BJN19790109

Czarnecki, J. W. and Breckenridge, G. (1977). Design and development of a long-term rumen simulation technique (RUSITEC). *Brit J Nutr* 38, 371-384. doi:10.1079/BJN19770102

Czarnecki, J. W. and Breckenridge, G. (1979a). Experiments with the long-term rumen simulation technique (RUSITEC): Response to supplementation of the rations. *Brit J Nutr* 42, 217-228. doi:10.1079/BJN19790109

Czarnecki, J. W. and Breckenridge, G. (1979b). Experiments with the long-term rumen simulation technique (RUSITEC): Use of soluble food and an inert solid matrix. *Brit J Nutr* 42, 229-245. doi:10.1079/BJN19790110

Dacasto, M., Eeckhoute, C., Capolongoa, F. et al. (2005). Effects of rumen fermentation and ruminal microflora on the bioavailability of garlic oil and four of its compounds in sheep fed diets at different ruminal fermentation and ruminal microflora in different dairy cow genotypes. *J Dairy Sci* 97, 3906-3917. doi:10.3168/jds.2013-7708
fect of breed and gender on bovine liver cytochrome P450 3A (CYP3A) expression and inter-species comparison with other domestic ruminants. *Vet Res* 36, 179-190. doi:10.1051/vetres:2004066

Daniel, C. R., Labens, R., Argyle, D. et al. (2018). Extracorporeal perfusion of isolated organs of large animals – Bridging the gap between in vitro and in vivo studies. *ALTEX* 35, 77-98. doi:10.14573/altex.1611291

de Oliveira, A. S., de Oliveira, M. R. C., Campos, J. M. S. et al. (2010). In vitro ruminal degradation of ricin and its effect on microbial growth. *Anim Feed Sci Technol* 157, 4154. doi:10.1016/j.amsfeedsci.2010.01.006

De Vijlder, T., Valkenborg, D., Lemi`ere, F. et al. (2018). A tutorial in small molecule identification via electrospray ionization-mass spectrometry: The practical art of structural elucidation. *Mass Spectrom Rev* 37, 607-629. doi:10.1002/mas.21551

Dickinson, J., Cooke, M., King, R. et al. (1976). Milk transfer of pyrrolizidine alkaloids in cattle. *J Am Vet Med Assoc* 169, 1192-1196.

Döring, B. and Petzinger, E. (2014). Phase 0 and phase III transport in organs: Combined concept of phases in xenobiotic transport and metabolism. *Drug Metab Rev* 46, 261-282. doi:10.3109/03602532.2014.882353

Doddareddy, M. R., Cho, Y. S., Koh, H. Y. et al. (2006). In-vitro-to-in vivo extrapolation (IVIVE) by PBTK modeling for animal free risk assessment approaches of potential endocrine-disrupting compounds. *Arch Toxicol* 93, 401-416. doi:10.1007/s00204-018-2372-z

Fabian, E., Gomes, C., Birn, B. et al. (2019). In vitro-to-in vivo extrapolation (IVIVE) by PBTK modeling for animal free risk assessment approaches of potential endocrine-disrupting compounds. *Arch Toxicol* 93, 401-416. doi:10.1007/s00204-018-2372-z

Forsthuber, M., Kaiser, A. M., Granitzer, S. et al. (2019). Species-specific differences in the in vitro metabolism of lasiocarpine. *Chem Res Toxicol* 28, 2034-2044. doi:10.1021/acs.chemrestox.5b00253

Gerhardt, S. and Schmickel, M. (2016). Isolation and cultivation of adult primary bovine hepatocytes from abattoir derived liver. *EXCLI J* 15, 858-866. doi:10.17179/excli2016-794

Endo, S., Brown, T. N. and Goss, K. U. (2013). General model for estimating partition coefficients to organisms and their tissues using the biological compositions and polypeptide linear free energy relationships. *Environ Sci Technol* 47, 6630-6639. doi:10.1021/es401772m

Engstrakul, J. J., Foti, R. S., Strelevitz, T. J. et al. (2005). Altered AZT (3′-azido-3′-deoxythymidine) glucuronidation kinetics in liver microsomes as an explanation for underprediction of in vivo clearance: Comparison to hepatocytes and effect of incubation environment. *Drug Metab Dispos* 33, 1621-1627. doi:10.1124/dmd.105.005058

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.

Forsthuber, M., Kaiser, A. M., Granitzer, S. et al. (2020). Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. *Environ Int* 137, 105324. doi:10.1016/j.envint.2019.105324

Francoise Domingue, B. M., Dellow, D. W., Wilson, P. R. et al. (1991). Comparative digestion in deer, goats, and sheep. *NZ J Agric Res* 34, 45-53. doi:10.1080/00288233.1991.10417792

Gabel, M., Pieper, B., Friedel, K. et al. (2003). Influence of nutrition level on digestibility in high yielding cows and effects on energy evaluation systems. *J Dairy Sci* 86, 3992-3998. doi:10.3168/jds.S0022-0302(03)74010-7

Gallo, A., Giuberti, G., Bertuzzi, T. et al. (2013). General modeling of pyrrolizidine alkaloids in cattle. *Am J Vet Res* 65, 243-254. doi:10.1161/ajp.2014.1909131

Gerhardt, S. and Schmickel, M. (2016). Isolation and cultivation of adult primary bovine hepatocytes from abattoir derived liver. *EXCLI J* 15, 858-866. doi:10.17179/excli2016-794

Endo, S., Brown, T. N. and Goss, K. U. (2013). General model for estimating partition coefficients to organisms and their tissues using the biological compositions and polypeptide linear free energy relationships. *Environ Sci Technol* 47, 6630-6639. doi:10.1021/es401772m

Engstrakul, J. J., Foti, R. S., Strelevitz, T. J. et al. (2005). Altered AZT (3′-azido-3′-deoxythymidine) glucuronidation kinetics in liver microsomes as an explanation for underprediction of in vivo clearance: Comparison to hepatocytes and effect of incubation environment. *Drug Metab Dispos* 33, 1621-1627. doi:10.1124/dmd.105.005058

Fabian, E., Gomes, C., Birn, B. et al. (2019). In vitro-to-in vivo extrapolation (IVIVE) by PBTK modeling for animal free risk assessment approaches of potential endocrine-disrupting compounds. *Arch Toxicol* 93, 401-416. doi:10.1007/s00204-018-2372-z

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.

Fashe, M. M., Juvonen, R. O., Petsalo, A. et al. (2015). Specificity of azithromycin metabolism in human liver microsomes. *J Biol Chem* 290, 1312-1320.
Grosse-Siestrup, C., Fehrenberg, C., von Baeyer, H. et al. (2002). Multiple-organ harvesting for models of isolated hemoperfused organs of slaughtered pigs. *ALTEX 19*, 9-13. https://www.altext.org/index.php/altext/article/view/1113

Grosse-Siestrup, C., Unger, V., Meissler, M. et al. (2003). Hemoperfused isolated porcine slaughterhouse kidneys as a valid model for pharmacological studies. *J Pharm Sci 92*, 1147-1154. doi:10.1010/jps.10383

Guengerich, F. P. (1997). Comparisons of catalytic selectivity of cytochrome P450 subfamily enzymes from different species. *Chem Biol Interact 106*, 161-182. doi:10.1016/S0099-2797(97)00068-9

Gunberg, D. L., Lyons, W. R. and Johnson, R. E. (1955). Perfusion studies of the isolated young rat liver. *J Lab Clin Med 45*, 130-134. doi:10.5555/uri:0022214355900627

Hahn, I., Kunz-Vekiru, E., Twarużek, M. et al. (2015). Aerobic and anaerobic in vitro testing of feed additives claiming to detoxify deoxynivalenol and zearalenone. *Food Addit Contam A 32*, 922-933. doi:10.1080/19440049.2015.1023741

Halwachs, S., Wassermann, L., Lindner, S. et al. (2013). Fungi cide prochloraz and environmental pollutant dioxin induce the ABCG2 transporter in bovine mammary epithelial cells by the arylhydrocarbon receptor signaling pathway. *Toxicol Sci 131*, 491-501. doi:10.1093/toxsci/kfs304

Hannah, S. M., Stern, M. D. and Ehle, F. R. (1986). Evaluation of a dual flow continuous culture system for estimating bacterial fermentation in vivo of mixed diets containing various soybean products. *Anim Feed Sci Technol 16*, 51-62. doi:10.1016/0377-8401(86)90049-0

Harms, M. J., Birch, R., Martens, H. et al. (1973). The effect of Strongyloides ransomi-infection on intestinal amino acid absorption in piglets. *Parasitenk 41*, 47-60. doi:10.1007/BF00329629

Hartinger, T., Gresner, N. and Südekum, K.-H. (2018). Comparison of differential solid-liquid removal rates on protozoa numbers in continuous cultures of rumen contents. *J Anim Sci 43*, 528-534. doi:10.2527/jas1976.432528x

Huang, W. and Isoherranen, N. (2018). Development of a dynamic physiologically based mechanistic kidney model to predict renal clearance. *CPT Pharmacometrics Syst Pharmacol 7*, 593-602. doi:10.1002/psp4.12321

Hubbard, S. A., Brooks, T. M., Gonzalez, L. P. et al. (1985). Preparation and characterisation of 59 fractions. In J. M. Parry and C. F. Arlett (eds.), *Comparative Genetic Toxicology: The Second UKEMS Collaborative Study*. London, UK: Palgrave Macmillan. doi:10.1007/978-1-349-07901-8_50

Huws, S. A., Creevey, C. J., Oyama, L. B. et al. (2018). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: Past, present, and future. *Front Microbiol 9*, 2161. doi:10.3389/fmicb.2018.02161

Huber, R. and Titajew, A. (1930). Über die Sekretionsarbeit der Leber vom Frosch. *Pflügers Arch 223*, 180-194. [Article in German]. doi:10.1007/BF01794080

Hoogenboom, L. A., Mulder, P. P., Zeilmaker, M. J. et al. (2011). Multiple-organ harvesting for models of isolated hemoperfused organs of slaughtered pigs. *ALTEX 38(3)*, 47-60.

Höber, R. and Titajew, A. (1930). Über die Sekretionsarbeit der Leber vom Frosch. *Pflügers Arch 223*, 180-194. [Article in German]. doi:10.1007/BF01794080

Hoogenboom, L. A., Mulder, P. P., Zeilmaker, M. J. et al. (2011). Carry-over of pyrrolizidine alkaloids from feed to milk in dairy cows. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess 28*, 359-372. doi:10.1080/19440049.2010.547521

Hubbard, S. A., Brooks, T. M., Gonzalez, L. P. et al. (1985). Preparation and characterisation of 59 fractions. In J. M. Parry and C. F. Arlett (eds.), *Comparative Genetic Toxicology: The Second UKEMS Collaborative Study*. London, UK: Palgrave Macmillan. doi:10.1007/978-1-349-07901-8_50

Huws, S. A., Creevey, C. J., Oyama, L. B. et al. (2018). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: Past, present, and future. *Front Microbiol 9*, 2161. doi:10.3389/fmicb.2018.02161

James, A. T., Peeters, G. and Lauryssens, M. (1956). The metabolism of propionic acid. *Eur J Clin Pharmacol 44*, 513-519. doi:10.1007/BF02440850

Jeong, J. S., Lee, J. H., Simizu, Y. et al. (2010). Effects of the Fusarium mycotoxin deoxynivalenol on in vitro rumen fermentation. *Anim Feed Sci Technol 162*, 145-153. doi:10.1016/j.anifeedsci.2010.09.009

Janku, I. (1993). Physiological modelling of renal drug clearance. *Eur J Clin Pharmacol 44*, 513-519. doi:10.1007/BF02440850

Jeong, J. S., Lee, J. H., Simizu, Y. et al. (2010). Effects of the Fusarium mycotoxin deoxynivalenol on in vitro rumen fermentation. *Anim Feed Sci Technol 162*, 144-148. doi:10.1016/j.anifeedsci.2010.09.009

Jia, L. and Liu, X. (2007). The conduct of drug metabolism studies considered good practice (II): In vitro experiments. *Carr Drug Metab 8*, 822-829. doi:10.2174/138920007782798207

Johnson, A. E. (1976). Changes in calves and rats consuming milk from cows fed chronic lethal doses of Senecio jacobaea (tansy ragwort). *Am J Vet Res 37*, 107-110.

Jones, H. M. and Houston, J. B. (2004). Substrate depletion approach for determining in vitro metabolic clearance: Time dependencies in hepatocyte and microsomal incubations. *Drug Metab Dispos 32*, 973-982. doi:10.1124/dmd.104.000125

Kedderis, G. L. (2018). Toxicokinetics: Biotransformation of fatty acids (SCFA) in the porcine proximal colon are influenced by SCFA concentration and luminal pH. *Comp Biochem Physiol A Mol Integr Physiol 158*, 169-176. doi:10.1016/j.cbpa.2010.10.018

Hindrichsen, I. K., Wettstein, H.-R., Machmüller, A. et al. (2004). Effects of feed carbohydrates with contrasting properties on rumen fermentation and methane release in vitro. *Can J Anim Sci 84*, 265-276. doi:10.4141/A03-095
toxicants. In C. A. McQueen (ed.), Comprehensive Toxicology. 3rd edition. Oxford, UK: Elsevier.

Kent-Dennis, C., Aschenbach, J. R., Griebel, P. J. et al. (2020). Effects of lipopolysaccharide exposure in primary bovine ruminal epithelial cells. J Dairy Sci 103, 9587-9603. doi: 10.3168/jds.2020-18652

Khiaosa-Ard, R., Bryner, S. F., Scheeder, M. R. L. et al. (2009). Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. J Dairy Sci 92, 177-188. doi:10.3168/jds.2008-1117

Kietzmann, M., Löscher, W., Arens, D. et al. (2015). Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. Appl Environ Microbiol 47, 1070-1073

Kietzmann, M., Löscher, W., Arens, D. et al. (2009). Changes in fibre-adherent and fluid-associated microbial communities and fermentation profiles in the rumen of cattle fed diets differing in hay quality and concentrate amount. FEMS Microbiol Ecol 93, 90-100

Klavenhusen, F., Petri, R. M., Kleefisch, M.-T. et al. (2017). Combination of α-linolenic acid biodegradation in the rumen simulation technique (RUSITEC)? Environ Sci Eur 27, 30. doi:10.1186/s12302-015-0063-4

Krebs, K. R. (2014). Invited review: Applied nutrition of ruminants: Fermentation and digestive physiology. Prof Anim Sci 30, 129-139. doi:10.15232/1S1080-7446(15)30100-5

Krishnamoorthy, U., Rymer, C. and Robinson, P. H. (2005). The in vitro gas production technique: Limitations and opportunities. Anim Feed Sci Technol 123-124, 1-7. doi:10.1016/j.anifeedsci.2005.04.015

Kroger, S., Pieper, R., Schwelberger, H. G. et al. (2013). Diets high in heat-treated soybean meal reduce the histamine-induced epithelial response in the colon of weaned piglets and increase epithelial catabolism of histamine. PLoS One 8, e0150115.

Kroger, S., Pieper, R., Aschenbach, J. R. et al. (2015). Effects of high levels of dietary zinc oxide on ex vivo epithelial histamine response and investigations on histamine receptor action in the proximal colon of weaned piglets. J Anim Sci 93, 5265-5272. doi:10.2527/jas.2015-9095

Kudo, H., Cheng, K.-J., Majak, W. et al. (1984). Degradation of mimosine in rumen fluid from cattle and sheep in Canada. Can J Anim Sci 64, 937-942. doi:10.4141/cjas84-105

Kuilmann, M. E. M., Maas, R. F. M. and Fink-Gremmels, J. (2000). Cytochrome P450-mediated metabolism and cytotoxicity of aflatoxin B1 in bovine hepatocytes. Toxicol In Vitro 14, 321-327. doi:10.1016/S0887-8333(00)00025-4

Kuroda, K., Kyiono, T., Isogai, E. et al. (2015). Immunization of fetal bovine colon epithelial cells by expression of human cyclin D1, mutant cyclin dependent kinase 4, and telomerase reverse transcriptase: An in vitro model for bacterial infection. PLoS One 10, e0143473. doi:10.1371/journal.pone.0143473

Larsson, P., Busk, L. and Tjälve, H. (1994). Hepatic and extrahepatic bioactivation and GSH conjugation of aflatoxin B1 in sheep. Carcinogenesis 15, 947-955. doi:10.1093/carcin/15.5.947

Laveé, T. and Funk, C. (2007). 5.03 – In vivo absorption, distribution, metabolism, and excretion studies in discovery and development. In J. B. Taylor and D. J. Trigg (eds.), Comprehensive Medicinal Chemistry II (31-50). Elsevier. doi:10.1016/B0-08-045044-X/00118-8

Leavens, T. L., Tell, L. A., Clothier, K. A. et al. (2012). Development of a physiologically based pharmacokinetic model to predict tulathromycin distribution in goats. J Vet Pharmacol Ther 35, 121-131. doi:10.1111/j.1365-8785.2011.01304.x

Lengowski, M. B., Zuber, K. H. R., Witzig, M. et al. (2016). Changes in rumen microbial community composition during adaption to an in vitro system and the impact of different forages. PLoS One 11, e0150115. doi:10.1371/journal.pone.0150115

Lescoat, P., Sauvant, D. and Danfaer, A. (1996). Quantitative aspects of blood and amino acid flows in cattle. Reprod Nutr Dev 36, 137-174. doi:10.1051/rnd:19960202

Lewis, K. A., Tzilivakis, J., Green, A. et al. (2013). Review of substances/agents that have direct beneficial effect on the environment: Mode of action and efficacy. EFSA Supporting Publication 10, 440E. doi:10.2903/sp.efsa.2013.EN-440

Li, S., Teng, L., Liu, W. et al. (2017a). Interspecies metabolic diversity of harmaline and harmine in vitro 11 mammalian liver microsomes. Drug Test Anal 9, 754-768. doi:10.1002/dta.2028

Li, M., Gehring, R., Riviere, J. E. et al. (2017b). Development
and application of a population physiologically based pharmacokinetic model for penicillin G in swine and cattle for food safety assessment. *Food Chem Toxicol* 107, 74-87. doi:10.1016/j.fct.2017.06.023

Li, M., Gehringer, R., Riviere, J. E. et al. (2018). Probabilistic physiologically based pharmacokinetic model for penicillin G in milk from dairy cows following intramammary or intramuscular administrations. *Toxicol Sci* 164, 85-100. doi:10.1093/toxsci/kfy067

Lin, Z., Gehringer, R., Mochel, J. P. et al. (2016). Mathematical modeling and simulation in animal health – Part II: Principles, methods, applications, and value of physiologically based pharmacokinetic modeling in veterinary medicine and food safety assessment. *J Vet Pharmacol Ther* 39, 421-438. doi:10.1111/jvpt.12311

Lin, L. and Wong, H. (2017). Predicting oral drug absorption: Mini review on physiologically-based pharmacokinetic models. *Pharmaceutics* 9, 41. doi:10.3390/pharmaceutics9040041

Lin, L., Li, M., Wang, Y.-S. et al. (2020). Physiological parameter values for physiologically based pharmacokinetic models in food-producing animals. Part I: Cattle and swine. *J Vet Pharmacol Ther* 43, 385-420. doi:10.1111/jvpt.12861

Lindner, S., Halwachs, S. and Wassermann, L. (2013). Expression and subcellular localization of efflux transporter ABCG2/BCRP in important tissue barriers of lactating dairy cows, sheep and goats. *J Vet Pharmacol Ther* 36, 562-570. doi:10.1111/jvpt.12045

Liu, X., Huang, J., Sun, Y. et al. (2013). Identification of multiple binding sites for substrate transport in bovine organic anion transporting polypeptide 1a2. *Drug Metab Dispos* 41, 602-607. doi:10.1124/dmd.112.047910

Loccisano, A. E., Longnecker, M. P., Campbell Jr., J. L. et al. (2013). Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A* 76, 25-57. doi:10.1080/15287394.2012.722523

Loebell, C. E. (1849). De conditionibus quibus secretiones in glandulis perficiuntur. Dissertatio Inauguralis Marburg.

MacLachlan, D. J. (2009). Influence of physiological status on residues of lipophilic xenobiotics in livestock. *Food Addit Contam A* 26, 692-712. doi:10.1080/0265203080269170

MacManus-Spencer, L. A., Tse, M. L., Hebert, P. C. et al. (2010). Binding of perfluorocarboxylates to serum albumin: A comparison of analytical methods. *Anal Chem* 82, 974-981. doi:10.1021/ac90238u

Mahnke, H., Ballent, M., Baumann, S. et al. (2016). The ABCG2 efflux transporter in the mammary gland mediates veterinary drug secretion across the blood-milk barrier into milk of dairy cows. *Drug Metab Dispos* 44, 700-708. doi:10.1124/dmd.115.068940

Majak, W. and Cheng, K.-J. (1987). Hydrolysis of the cyanogentic glycosides amygdalin, prunasin and linamarin by ruminal microorganisms. *Can J Anim Sci* 67, 1133-1137. doi:10.4141/cjas87-120

Mansfield, H. R., Endres, I. E. and Stern, M. D. (1995). Comparison of microbial fermentation in the rumen of dairy cows and dual flow continuous culture. *Anim Feed Sci Technol* 55, 47-66. doi:10.1016/0377-8401(95)98202-8

Marin, J. J. G. (2012). Plasma membrane transporters in modern liver pharmacology. *Scientifica* 2012, 428139. doi:10.6064/2012/428139

Martínez, M. E., Ranilla, M. J., Tejido, M. L. et al. (2010a). Comparison of fermentation of diets of variable composition and microbial populations in the rumen of sheep and Rusitec fermenters. I. Digestibility, fermentation parameters, and microbial growth. *J Dairy Sci* 93, 3684-3698. doi:10.3168/jds.2009-2933

Martínez, M. E., Ranilla, M. J., Tejido, M. L. et al. (2010b). Comparison of fermentation of diets of variable composition and microbial populations in the rumen of sheep and Rusitec fermenters. II. Protozoa population and diversity of bacterial communities. *J Dairy Sci* 93, 3699-3712. doi:10.3168/jds.2009-2934

Martínez, M. N., Court, M. H., Fink-Gremmels, J. et al. (2018). Population variability in animal health: Influence on dose-exposure-response relationships: Part I: Drug metabolism and transporter systems. *J Vet Pharmacol Ther* 41, E57-E67. doi:10.1111/jvpt.12670

Mattocks, A. (1986). *Chemistry and Toxicology of Pyrrolizidine Alkaloids*. London, UK: Academic Press.

Maul, R., Warth, B., Kant, J. S. et al. (2012). Investigation of the hepatic glucuronidation pattern of the fusarium mycotoxin deoxynivalenol in various species. *Chem Res Toxicol* 25, 2715-2717. doi:10.1021/tr300348x

McAllister, T. A., Bae, H. D., Jones, G. A. et al. (1994). Microbial attachment and feed digestion in the rumen. *J Anim Sci* 72, 3004-3018. doi:10.2527/1994.72113004x

McDougall, E. J. (1948). Studies on ruminant saliva. I. The composition and output of sheep’s saliva. *Biochem J* 43, 99-109.

Miettinen, H. and Setälä, J. (1989). Design and development of a microcellular fermentation fermenter. II. Protozoa population and diversity of bacteriological contents. *J Dairy Sci* 93, 74-87. doi:10.3168/jds.2009-2934

Mojtahedi, M., Danesh Mesgaran, M., Vakili, S. A. et al. (2013). Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A* 76, 25-57. doi:10.1080/15287394.2012.722523

Moe, K., Hondo, T., Kanaya, T. et al. (2010). Characterization of newly established bovine intestinal epithelial cell line. *Histochem Cell Biol* 133, 125-134. doi:10.1007/s00418-009-0648-3

Mocchegiani, E. and Muzzalupo, G. (2011). Association of dietary polyphenols with prevention and treatment of metabolic syndrome: A review on human studies. *CNS Neurosci Ther* 17, 100-122. doi:10.1111/j.1755-5914.2011.00166.x

Mohan, V. and Bhat, H. K. (2012). Chemopreventive potential of_ _Bacopa monnieri_ _Linn_ _Brahmi_ _for_ _prevention_ _of_ _cancer_ _and_ _related_ _diseases. J Ethnopharmacol_ _138(3), 877-892._

Mohan, V. and Bhat, H. K. (2012). Chemopreventive potential of_ _Bacopa monnieri_ _Linn_ _Brahmi_ _for_ _prevention_ _of_ _cancer_ _and_ _related_ _diseases. J Ethnopharmacol_ _138(3), 877-892._

Mowlaei, M., Mokhtari, M., Ejhadizadeh, M. et al. (2012). Effect of ferulic acid on serum levels of rheumatoid arthritis patients. *J Ethnopharmacol* 142, 451-457. doi:10.1016/j.jep.2012.08.006

Morgavi, D., Boudra, H., Jouany, J.-P. et al. (2003). Prevention of patulin toxicity on rumen microbial fermentation by SH-containing reducing agents. *J Agric Food Chem* 51, 6906-6910. doi:10.1021/jf034505v
Morgavi, D., Boudra, H., Jouany, J.-P. et al. (2004). Effect and stability of gliotoxin, an Aspergillus fumigatus toxin, on in vitro rumen fermentation. *Food Addit Contam* 21, 871-878. doi:10.1080/02657760410001344
Morgavi, D., Forano, E., Martin, C. et al. (2010). Microbial ecosystem and methanogenesis in ruminants. *Animal* 4, 1024-1036. doi:10.1017/S1751731110000546
Morgavi, D., Martin, C. and Boudra, H. (2013). Fungal secondary metabolites from Monascus spp. reduce rumen methane production in vitro and in vivo. *J Anim Sci* 91, 848-860. doi:10.2527/jas.2012-5665
Moumen, A., Yañez-Ruiz, D. R., Carro, M. D. et al. (2019). Mechanisms and regulation of epithelial phosphate transport in ruminants: approaches in comparative physiology. *Pflügers Arch* 471, 185-191. doi:10.1007/s00424-018-2181-5
Nicken, P., Schröder, B., von Keutz, A. et al. (2016). Investigations on feed intake and digestibility of four domestic ruminant species eating leaf and stem fractions of grasses. *J Anim Sci* 91, 895-904. doi:10.1093/kjani/ksw062
Numata, J., Kowalczyk, J., Adolphs, J. et al. (2014). Toxicokinetic and metabolic stability of gliotoxin, an Aspergillus fumigatus toxin, on in vitro. *Kidney Int* 7(7), 1203-1253. doi:10.1016/j.kint.2013.12.025
Nizet, A. (1975). The isolated perfused kidney: Possibilities, limitations and results. *Kidney Int* 7, 1-11. doi:10.1038/ki.1975.1
NRC – National Research Council (1988). *Designing Foods: Animal Product Options in the Marketplace*. National Research Council (US) Committee on Technological Options to Improve the Nutritive Attributes of Animal Products. Washington, DC, USA: National Academies Press. doi:10.17226/1036
Nüdt, V., Marenz, O., Teyssier, O. et al. (2017). The influence of diet on the concentration of rumen protozoa in the ovine. *Austr J Agric Res* 32, 1344-1352. doi:10.1002/rcm.8173
OECDC (2007b). Test No. 503: Metabolism in Livestock. *OECD Guidelines for the Testing of Chemicals, Section 5. OECD Publishing*, Paris. doi:10.1080/004982598238886
Paini, A., J. A., Joosens, L. E., Besems, J. G. M. et al. (2019). Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. *Comput Toxicol* 9, 61-72. doi:10.1016/j.comtox.2018.11.002
Peeters, G. and Massart, L. (1952). Fat synthesis in the perfused lactating cow’s udder. *Arch Int Pharmacodyn Ther* 91, 389-398.
Peiners, K. (2004). Effect and metabolic stability. *OECD Guidelines for the Testing of Chemicals, No. 286*. OECD Publishing, Paris. doi:10.1787/9789264304796-en
Pelkonen, O., Mäeenpäeä, J., Taavitsainen, P. et al. (1998). Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica* 28, 1203-1253. doi:10.1080/004982598238886
Petri, R. M., Schwaiger, T., Penner, G. B. et al. (2013). Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. *Appl Environ Microbiol* 79, 3744-3755. doi:10.1128/AEM.03983-12
Petri, R. M., Mickdam, E., Klevenhusen, F. et al. (2019). Effects of the supplementation of plant-based formulations on microbial fermentation and predicted metabolic function in vitro. *Anaoerobe* 57, 19e27. doi:10.1016/j.anaoebe.2019.03.001
Pilarski, S. and Huisinga, W. (2010). Lumping of physiologically-based pharmacokinetic models and a mechanistic derivation of classical compartmental models. *J Pharmacokin Pharmacodynam* 37, 365-405. doi:10.1007/s10928-010-9165-1
Plattner, F. (1924). Zur Frage der Ausscheidung saurer Farbstoffe durch die Leber. *Pflügers Arch* 206, 91-100. doi:10.1007/BF01722753
Porp, D. P., Minson, D. J. and Ternouth, J. H. (1980). Studies of cattle and sheep eating leaf and stem fractions of grasses. 1. The voluntary intake, digestibility and retention time in the reticulo-rumen. *Austr J Agric Res* 32, 99-108. doi:10.1071/AR9810099
Potter, E. L. and Dehormy, B. A. (1973). Effects of changes in feed level, starvation, and level of feed after starvation upon the concentration of rumen protozoa in the ovine. *Appl Microbiol* 3, 692-698.
Poulin, P. and Theil, F. P. (2000). A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. *J Pharm Sci* 89, 16-35. doi:10.1002/sin.1150200010989.1:s1<16:AID-JPS3>3.0.CO;2-E
Riaz, M. Q., Südekum, K.-H., Clauss, M. et al. (2014). Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: A meta-analysis study. *Livest Sci* 162, 76-85. doi:10.1016/j.livsci.2014.01.009
Richardson, S. J., Bai, A., Kulkarni, A. A. et al. (2016). Efficiency in drug discovery: Liver S9 fraction assay as a screen for metabolic stability. *Drug Metab Lett* 10, 83-90. doi:10.2174/187231281066616023121836
Riede, S., Toboldt, A., Breves, G. et al. (2016). Investigations on the possible impact of a glycosylate-containing herbicide on
ruminal metabolism and bacteria in vitro by means of the ‘Rumen Simulation Technique’. J Appl Microbiol 121, 644-656. doi:10.1111/jam.13190
Roeder, E. (2000). Medicinal plants in China containing pyrrolizidine alkaloids. Pharmazie 55, 711-726.
Rosa, B. (2020). Equine drug transporters: A mini-review and veterinary perspective. Pharmaceutics 12, 1064. doi:10.3390/pharmaceutics12111064
Rozehnal, V., Nakai, D., Hoepner, U. et al. (2012). Human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for characterizing the intestinal absorption of drugs. Eur J Pharm Sci 46, 367-373. doi:10.1016/j.ejps.2012.02.025
Russel, W. M. S. and Burch, R. L. (1959). The Principles of Humane Experimental Technique. University Federation for Animal Welfare UFAW, Potters Bar, England.
Rymer, C., Huntingdon, J. A., Williams, B. A. et al. (2005). In vitro cumulative gas production techniques: History, methodological considerations and challenges. Anim Feed Sci Technol 123-124, 9-30. doi:10.1016/j.anifeedssci.2005.04.055
Sanchez, R. I. and Kaufman, F. C. (2010). Regulation of xenobiotic metabolism in the liver. In C. A. McQueen (ed.), Comprehensive Toxicology. 2nd edition. Oxford, UK: Elsevier. doi:10.1016/B978-0-08-046884-6.01005-8
Savateeva, D., Numata, J., Pieper, R. et al. (2020). Physiologically based toxicokinetic models and in silico predicted partition coefficients to estimate tetrachlorodibenzo-p-dioxin transfer from feed into growing pigs. Arch Toxicol 94, 187-196. doi:10.1007/s00204-019-02617-0
Schmitt, W. (2008). General approach for the calculation of tetrachlorodibenzo-p-dioxin from feed into growing pigs. Arch Toxicol 92, 689-700. doi:10.1007/s00204-016-1881-x
Strobelt, E., Seeling, K. and Tebbe, C. C. (2008). Diversity responses of rumen microbial communities to Fusarium-contaminated feed, evaluated with rumen simulating technology. Environ Microbiol 10, 483-496. doi:10.1111/j.1462-2920.2007.01469.x
Stumpf, F., Martens, H., Bilk, S. et al. (2009). Cultured ruminal epithelial cells express a large-conductance channel permeable to chloride, bicarbonate, and acetate. Pflugers Arch – Eur J Physiol 457, 1003-1022. doi:10.1007/s00424-008-0566-6
Stumpf, F., Georgi, M. I., Mundhenk, L. et al. (2011). Sheep rumen and omasum primary cultures and source epithelia: Barrier function aligns with expression of tight junction proteins. J Exp Biol 214, 2871-2882. doi:10.1242/jeb.055582
Terry, S. A., Ramos, A. F. O., Holman, D. B. M. et al. (2018). Humic substances alter ammonia production and the microbial populations within a RUSITEC fed a mixed hay-concentrate diet. Front Microbiol 9, 1410. doi:10.3389/fmicb.2018.01410
Thiel, A., Rümbelie, R., Mair, P. et al. (2019). 3-NOP: ADMET studies in rats and ruminating animals. Food Chem Toxicol 125, 528-539. doi:10.1016/j.fct.2019.02.002
Tolonen, A. and Pelkonen, O. (2015). Analytical challenges for conducting rapid metabolism characterization for QIVIVE. Toxicology 332, 20-29. doi:10.1016/j.tox.2013.08.010
Udén, P., Robinson, P. H., Mateos, G. G. et al. (2012). Use of replicates in statistical analyses in papers submitted for publication in animal feed science and technology. Anim Feed Sci Technol 171, 1-5. doi:10.1016/j.anifeedssci.2011.10.008
Uppal, S. K., Wolf, K. and Martens, H. (2003). The effect of short chain fatty acids on calcium flux rates across isolated rumen epithelium of hay-fed and concentrate-fed sheep. J Anim Physiol Nutr 87, 12-20. doi:10.1046/j.1439-0396.2003.00401.x
Ussing, H. H. (1949). The active ion transport through the isolated frog skin in the light of tracer studies. Acta Physiol Scand 17, 1-37. doi:10.1111/j.1439-0442.1997.tb01137.x
Ussing, H. H. and Zerahn, K. (1951). Active transport of sodium as the source of electric current in the short-circuit isolated frog skin. Acta Physiol Scand 23, 110-127. doi:10.1111/j.1439-0442.1951.tb00550.x
Váradyová, Z., Mihaliková, K., Kišidayová, S. et al. (2006). Fermentation pattern of the rumen and hindgut inocula of sheep

Smith, D. J. and Shelver, W. L. (2002). Tissue residues of ractopamine and urinary excretion of ractopamine and metabolites in animals treated for 7 days with dietary ractopamine. J Anim Sci 80, 1240-1249. doi:10.2527/2002.8051240x
Solvíva, C. R., Meile, L., Cieslák, A. et al. (2004). Rumen simulation technique study on the interactions of dietary lauric and myristic acid supplementation in suppressing ruminal methanogenesis. Br J Nutr 92, 689-700. doi:10.1079/BJN20041250
Starling, E. H. and Verney, E. B. (1925). The secretion of urine as studied on the isolated kidney. Proc R Soc Lond B 97, 321-363. doi:10.1098/rspb.1925.0004
Strikwold, M., Spenkelink, B., de Haan, L. H. J. et al. (2017). Integrating in vitro data and physiologically based kinetic (PBK) modelling to assess the in vivo potential developmental toxicity of a series of phenols. Arch Toxicol 91, 2119-2133. doi:10.1007/s00204-016-1881-x

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grazing in an area polluted from the non-ferrous metal industry. *Czech J Anim Sci* 51, 66-72. doi:10.17221/3911-CJAS

Verbeke, R., Åqvist, S. and Peeters, G. (1957). Acetate and propionate as precursors of amino-acids in milk proteins of the perfused cow’s udder. *Arch Int Physiol Biochim* 65, 433-438. doi:10.3109/13813455709069427

Virkel, G., Bellent, M., Lanusse, C. et al. (2019). Role of ABC transporters in veterinary medicine: Pharmacologic-toxicological implications. *Curr Med Chem* 26, 1251-1269. doi:10.2174/0929867325666180201094730

Viviani, P., Lifschitz, A. L., García, J. P. et al. (2017). Assessment of liver slices for research on metabolic drug-drug interactions in cattle. * Xenobiotica* 47, 933-942. doi:10.1080/00958912.2016.12614782

von Jagow, R., Kampffmeyer, H. and Kinese, M. (1965). The preparation of microsomes. *Naunyn Schmiedebergs Arch* 251, 73-87. doi:10.1007/BF00245731

Wallace, R. J. and Newbold, C. J. (1991). Effects of bentonite on fermentation in the rumen simulation technique (Rusitec) and on rumen ciliate protozoa. *J Agric Sci* 116, 163-168. doi:10.1017/S0021859600076279

Watanabe, R., Esaki, T. et al. (2019). Development of an in silico prediction system of human renal excretion and clearance from chemical structure information incorporating fraction unbound in plasma as a descriptor. *Sci Rep* 9, 18782. doi:10.1038/s41598-019-55325-1

Wen, B. and Zhu, M. (2015). Applications of mass spectrometry in drug metabolism: 50 years of progress. *Drug Metab Rev* 47, 71-87. doi:10.1016/j.dmr.2014.1001029

Westlake, K., Mackie, R. I. and Dutton, M. F. (1989). In vitro metabolism of mycotoxins by bacterial, protozoal and ovine ruminal fluid preparations. *Anim Feed Sci Technol* 25, 169-178. doi:10.1016/0377-8401(89)90117-X

Wetzel, S. U., Mann, E., Metzler-Zebeli, B. U. et al. (2016). Epimural indicator phytophotes of transiently-induced subacute ruminal acidosis in dairy cattle. *Front Microbiol* 7, 274. doi:10.3389/fmicb.2016.00274

Wetzel, S. U., Eger, M., Burmester, M. et al. (2018). The application of rumen simulation technique (RUSITEC) for studying dynamics of the bacterial community and metabolome in rumen fluid and the effects of a challenge with *Clostridium perfringens*. *PLoS One* 13, e0192256. doi:10.1371/journal.pone.019225

Wiedenfeld, H. and Edgar, J. (2011). Toxicity of pyrrolizidine alkaloids to humans and ruminants. *Phytochem Rev* 10, 137-151. doi:10.1007/s11101-010-9174-0

Wilkins, M. R., Richter, J., Fraser, D. R. et al. (2012). In contrast to sheep, goats adapt to dietary calcium restriction by increasing intestinal absorption of calcium. *Comp Biochem Physiol A Mol Integr Physiol* 163, 396-406. doi:10.1016/j.cbpa.2012.06.011

Witte, S., Brockelmann, Y., Haeger, J. D. et al. (2019). Establishing a model of primary bovine hepatocytes with responsive growth hormone receptor expression. *J Dairy Sci* 102, 7522-7535. doi:10.3168/jds.2018-15873

Witzig, M., Boguhn, J., Zeder, M. et al. (2015). Effect of donor animal species and their feeding on the composition of the microbial community establishing in a rumen simulation. *J Appl Microbiol* 119, 33-46. doi:10.1111/jam.12829

Xiao, Y., Deng, J., Liu, X. et al. (2014). Different binding sites of bovine organic anion-transporting polypeptide1a2 are involved in the transport of different fluoroquinolones. *Drug Metab Dispos* 42, 1261-1267. doi:10.1124/dmd.114.057448

Yañez-Ruiz, D. R., Bannink, A., Dijkstra, J. et al. (2016). Design, implementation and interpretation of in vitro batch culture experiments to assess enteric methane mitigation in ruminants – A review. *Anim Feed Sci Technol* 216, 1-18. doi:10.1016/j.anifeedsci.2016.03.016

Yoshioka, M., Takeouchi, T., Kitani, H. et al. (2016). Establishment of SV40 large T antigen-immortalized bovine liver sinusoidal cell lines and their immunological responses to deoxyxynvalenol and lipopolysaccharide. *Cell Biol Int* 40, 1372-1379. doi:10.1002/cbin.10682

Zhan, K., Gong, X. X., Chen, Y. Y. et al. (2019). Short-chain fatty acids regulate the immune responses via G protein-coupled receptor 41 in bovine rumen epithelial cells. *Front Immunol* 10, 2042. doi:10.3389/fimmu.2019.02042

Zhang, M., van Ravenzwaay, B., Fabian, E. et al. (2018). Towards a generic physiologically based kinetic model to predict in vivo uterotrophic responses in rats by reverse dosimetry of in vitro estrogenicity data. *Arch Toxicol* 92, 1075-1088. doi:10.1007/s00204-017-2140-5

Zhang, K., Kurita, K. L., Venkatramani, C. et al. (2019). Seeking universal detectors for analytical characterizations. *J Pharm Biomed Anal* 162, 192-204. doi:10.1016/j.jpba.2018.09.029

Zhang, M., van Ravenzwaay, B. and Rietjens, I. M. C. M. (2020). Development of a generic physiologically based kinetic model to predict in vivo uterotrophic responses in rats based on reverse dosimetry of in vitro estrogenicity data. *Arch Toxicol* 92, 1075-1088. doi:10.1007/s00204-017-2140-5

Ziener, C. J., Sharp, R., Stern, M. D. et al. (2000). Comparison of microbial populations in model and natural rumens using 16S ribosomal RNA-targeted probes. *Environ Microbiol* 2, 632-643. doi:10.1046/j.1462-2920.2000.00146.x

Ziv, G. and Rasmussen, E. (1975). Distribution of labeled antibiotics in different components of milk following intramammary and intramuscular administrations. *J Dairy Sci* 58, 938-946. doi:10.3168/jds.S0022-0302(75)84660-1

Conflict of interest

The authors declare that they have no conflicts of interest.