REVIEW

# Defense mechanisms against toxic phytochemicals in the diet of domestic animals

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Plant secondary metabolites (PSMs) are non-nutritional components that occur in numerous feed materials and are able to exert toxic effects in animals. The current article aims to summarize innate defense strategies developed by different animal species to avoid excessive exposure to PSMs. These mechanisms include pre-systemic degradation of PSMs by rumen microbiota, the intestinal barrier including efflux transporters of monogastric species, as well as pre-hepatic and intra-hepatic biotransformation processes. These physiological barriers determine systemic exposure and ultimately the dose-dependent adverse effects in the target animal species. Considering the large number of potentially toxic PSMs, which makes an evaluation of all individual PSMs virtually impossible, such a mechanism-oriented approach could improve the predictability of adverse effects and support the interpretation of clinical field observations. Moreover, mechanistic data related to tissue disposition and excretion pathways of PSMs for example into milk, could substantially support the assessment of the risks for consumers of foods derived from PSM-exposed animals.

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## 1 Introduction

Phytochemicals are defined as non-nutritive plant metabolites, which are produced by plants in response to environmental stress conditions and in response to injury following the invasion by phytopathogenic agents such as viruses, bacteria, fungi, or herbivorous insects [1–4]. The defense response is coordinated by a complex network of plant hormones, which activate specific transcription factors in the plant genome. This innate immune response is linked to biosynthetic pathways that are involved in the production of individual classes of plant secondary metabolites (PSMs) [5, 6]. Major biosynthetic pathways are associated with the availability of acetyl-CoA, which is involved in the formation of anthraquinones, or shikimic acid serving as building block for alkaloids and phenylpropanoids such as lignans, aromatic essential oils, and coumarins. Mevalonate and deoxycellulose pathways lead to the synthesis of terpenoids and steroid metabolites including many saponins, terpenoid essential oils, and carotinoids [7]. The biological activity of PSMs is diverse and includes attractant and deterrent activity to insects and herbivorous mammals, as well anti-viral, anti-bacterial, and anti-fungal effects.

Grasses and sedges tolerate herbivorous animals as their basal meristem facilitates rapid re-growth. Subsequently, these monocotyledons form only a small number of secondary metabolites. Well-known examples are the synthesis of avenacins (antibacterial triterpene glycosides, belonging to the group of saponins) by *Avena* species, including oats [8, 9] and tannins [10]. In addition, various grasses are protected by symbiotic endophytes producing

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**Abbreviations: CAR,** constitutive androstane receptor; **Nrf2,** nuclear factor-erythroid 2 p45-related factor 2; **PSM,** plant secondary metabolite; **PXR,** pregnane X receptor; **UGT,** UDP-glucuronosyl transferase

essential insect repellents such as pyramine. Pyramine is a loline derivative, and the same biosynthetic pathway results in more complex chemical structures, as for example the group of lolitrems, which exert neurotoxic effects in grazing animals (rye grass staggers) or ergot alkaloids, affecting animal health and reproduction (tall fescue toxicosis) [11, 12]. Pasture grasses can also be invaded by numerous other toxinogenic moulds, particularly by *Fusarium* species [12, 13].

Dicotyledons use PSMs in the form of colorants and volatile compounds to attract insects but also as protective agents against deterioration by grazing mammals [14]. In a natural environment, animals recognize these toxic plants and avoid their consumption. However, when plants (or animals) are transferred to non-endemic regions or plants are harvested and dried, this recognition is often lost. Moreover, the use of by-products from plant oil production or distilling processes as feed materials can be associated with undesirable high exposure of farm animals to PSMs and hence with anti-nutritive or toxic effects [15].

PSMs have been studied for many centuries, both as toxic substances as well as therapeutic agents [7]. The number of potentially toxic plant metabolites is large, exceeding 100 000 individual substances (Table 1), and taken together they can affect virtually any function of a living organism. Prominent examples for chemical classes of PSMs that occur in animal feeds and are related to clinical signs of intoxications are the alkaloids (tropane alkaloids as well as pyrrolizidine alkaloids), glucosinolates, terpenes, and saponines, as well as flavonoids.

It is beyond the scope of a single review to describe all chemical properties and the biological activity of all currently known PSMs that may affect the health and productivity of farm animals [16, 17]. Instead, the current review will focus on the physiological defense mechanisms that mammals have developed during their evolution to protect themselves against toxic plant metabolites.

**Table 1.** Major classes of plant secondary metabolites (modified [7])

| Chemical class | Estimated number of products |
|---|---|
| Alkaloids | 12 000 |
| Non-protein amino acids | 600 |
| Amines | 100 |
| Cyanogenic glycosides | 100 |
| Glucosinolates | 600 |
| Monoterpenes | 1000 |
| Sesquiterpenes | 3000 |
| Diterpenes | 2000 |
| Triterpenes, Saponines, Steroids | 4000 |
| Flavonoids | >2000 |
| Polyacetylenes | 1000 |
| Polyketides | 750 |
| Phenylpropanes | 1000 |

## 2 Polygastric herbivory: Pre-systemic detoxification of phytochemicals by the rumen microbiota

The most effective system to utilize diverse, even low-quality plant materials is found in ruminants. The rumen flora consists of a complex community of microorganisms, of which bacteria and protozoa are the major classes. Their physiological function is the degradation of cellulose, hemicelluloses, pectin, and other complex fibers, which in turn are used for the production of volatile fatty acids and microbial proteins. These products are utilized by the mammalian host to fulfill its nutritive demands. The rumen microbiota shows a species-specific composition, which is unique (finger-print) for the individual animal. Individual populations of microorganisms can be affected temporarily by dietary components [18].

It is generally assumed that the rumen microorganisms are able to hydrolyze and deactivate virtually all toxic plant metabolites, thus protecting the animal. This assumption is based on clinical observations indicating that ruminants are indeed less susceptible to many PSMs when compared with (monogastric) horses, which often consume comparable roughages. However, details about the actual biotransformation pathways used by rumen microbiota to convert potentially toxic PSMs are often lacking and may involve biochemical reactions, which can be performed only by some microorganisms. One of the most prominent examples that demonstrate this high specificity is the degradation of mimosine, a metabolite of the tropical shrub leucanea (*Leucanea leucocephala*) [19]. Leucenea is a persistent legume originating from Central America now being spread to many other continents. Clinical reports described intoxications in animals in non-endemic areas, whereas in endemic areas the plant was well tolerated by ruminants. Investigations into the rumen stability of mimosine, the major PSM, revealed that some rumen bacteria convert this compound into 3-hydroxy-4-(1H) pyridone, a strong goitrogen. When affected animals in non-endemic areas were inoculated with rumen fluid from endemic areas, tolerance to leucaena (and mimosin) could be achieved [20]. Further investigations showed that the critical step in the bacterial biotransformation is conducted by an individual 3-hydroxy-4-(1H) pyridone-degrading organism (*Synergistes jonesii*), which commonly occurs in the rumen flora in endemic regions. When *Synergistes* was inoculated in a few animals of a herd, it rapidly spread to all animals and conferred tolerance to leucaena PSMs. The insight in this mechanism allowed the use of leucaena, a high-quality legume, as forage plant in tropical and subtropical regions [21].

Glucosides of 3-nitro-1 propanol (nitropropanol) and glucose esters of 3-nitro-1 propanoic acid, which occur in many *Astragalus* species, comprise a group of nitrotoxins that, following hepatic activation, act as potent enzyme inhibitors inactivating cellular succinate dehydrogenase and subsequently ATP formation, resulting in clinical signs of

 

intoxication such as dyspnoea, muscular incoordination, and depression and weight loss [22]. Tolerance of grazing animals depends on the capacity of the rumen flora to degrade the nitrotoxins prior to absorption, a process that is mainly catalyzed by the rumen bacterium *Denitrobacterium detoxificans*. Feeding strategies, such as addition of soybean proteins to the animal's diet, increase the density of *D. detoxificans* in the rumen and hence the detoxicification capacity, thereby increasing the tolerance of animals to nitrotoxins present in forages [22].

Saponins are found in many plant species, comprising a group of glycosides with a triterpene or steroidal aglycone-moiety, which is linked to one or more sugar chains [10]. Saponins have been intensively studied in ruminants due to their potential anti-protozoal activity, which might increase animal performance, particularly in ruminants fed a low protein diet. Prominent examples for saponin-producing plants are alfalfa [23], *Yucca schidegera* [24], *Acacia auriculoformis* [25], and fenugreek seeds [26]. Their utilization depends on the ingested amount (dose) but also on the composition of the diet, and hence both beneficial and toxic effects have been reported.

Glucosinolates, a large group of compounds characterized by a β-d-thioglucose group linked to variable side chains, consisting of amino acids such as methionine, tryptophan, or phenylalanine are the major metabolites of *Brassica* species [27, 28]. Following the rupture of plant structures, for example by harvesting or chewing, plant-derived β-thioglucosidase (myrosinase, which in the plant is sequestered in vesicles) converts the non-toxic glucosinolates into instable aglucons, which are further metabolized by different pathways into isothiocyanates, oxazolidinethiones (5-vinyl-2-oxazolidinethione and 5-vinyl-1,3 oxazolidine-2-thione), thiocyanates, nitriles, epithionitriles, and other toxic indol-3-ylmethyl derivatives [29]. The toxicity of glucosinolates depends predominantly on the formation of thiocyanates, oxazolidines, and nitriles. These compounds interfere with iodine uptake (thiocyanates) and the synthesis of the thyroid hormones $T_3$ and $T_4$, (oxazolidinethiones), leading eventually to hypothyroidism and enlargement of the thyroid gland (goiter) [30]. In ruminants the initial hydrolysis of the glucosinolates can also be performed by bacterial β-glucosidases. However, as the same microorganisms degrade the toxic oxazolidines and (epithio)nitriles, ruminants are still more tolerant to *Brassica* toxins than monogastric herbivores [31].

Like other complex groups of PSMs, polyphenolic tannins can exert beneficial effects as well as adverse reactions, depending on the animal species and the chemical nature and concentrations in feeds [32, 33]. Tannins occur in two distinct forms, denoted as hydrolysable tannins, which are derivatives of gallic acid, which can be partly esterified to a polyol such as glucose, glucitol, quercitol, or shikimic acid, and condensed tannins, comprising proanthocyanidines that are either di-, tri-, or polymers of anthocyanidins and/or catechin-flavan-3-ol, or leucoanthocyanides that are dimers of flavones-3,4-di-ol flavonoids. As the monomers can assemble to a virtually infinite number of oligomers, the biological properties vary according to the chemical structure [34, 35]. In general, tannins have been regarded as inhibitory to the rumen microbiota [36]. However, different tannins have also different effects on individual microbial species, apparently related to their molecular weight [37]. This applies also to their anti-protozoal activity, as tannins from certain plants reduce the total number of rumen protozoa [38], while others seem to have no effect [39] or even increase protozoal numbers, such as tannins from Acacia leaves, sulla (*Hedysarum coronarium* L.) or birdsfoot trefoil (*Lotus corniculatus*) [40]. Moderate amounts of condensed tannins have shown beneficial effects in stabilizing rumen pH followed by an increased milk yield in ewes [41].

Recently, plant-derived essential oils, generally consisting of diverse phenylpropenes and terpenes, have gained increasing interest in animal nutrition due to their antibiotic activity. These effects have been known for centuries and are used in traditional food preservation in the form of herbs and spices [42]. These compounds are successfully used in monogastric species, to stabilize their gut flora [43]. In ruminants, their antibiotic activity can be detrimental to the rumen flora. Indeed, Busquet *et al.* [44], when testing plant extracts and isolated PSMs, identified anethol, anise oil, carvane, and tea-tree oil as compounds that decrease rumen acetate and propionate production, making them nutritionally non-beneficial to dairy cattle. Diverging effects of PSMs on rumen flora fatty acid production were also measured at different pH conditions [45].

These few examples should demonstrate that the capacity of the rumen flora to detoxify secondary plant metabolites is not a universal trait, but is often related to individual rumen micro-organisms or the overall, concentration-dependent, detoxification capacity. The rumen flora represents also a target for various PSMs, as demonstrated for many essential oils. At present, various lines of research focus on the modulation of the rumen flora to increase its degradation capacity as this would allow a broader use of legumes and other potentially toxic plants as feed materials and probably reduce an undesirable overproduction of methane in large ruminants [36, 45].

## 3 Monogastric herbivory: Are acquired feeding strategies sufficiently protective?

In monogastric herbivores fermentation of plant cellulose and fibers occurs in the large intestines, for example in the caecum of equidae and avian species. As small sufficiently polar plant metabolites may pass the stomach unaffected and are rapidly absorbed in the small intestine, these animals need to select their diets more carefully. Insight

 

into specific defense mechanisms of monogastric animals originate from studies with wild herbivores, of which approximately 1% are dietary specialists [46], consuming plants with a very high concentration of PSMs. The most commonly known example is the Koala (*Phasolarctos cinereus*), which feeds exclusively on eucalyptus trees *(Eucalyptus punctata)*.

The best studied herbivore specialist, however, is the common woodrat, which occurs in North America in two varieties: *Neotoma albigula*, a dietary generalist, and *Neotoma stephensi*, a dietary specialist, which feeds more or less exclusively on juniper plants [46, 47]. These plants contain high amounts of α-pinene, a terpenoid with known toxicity in many herbivorous species. Observational studies revealed that *Neotoma stephensi* has developed a specific feeding strategy in that the animals eat only very small quantities of plant material *per* individual meal, thus avoiding peak plasma and toxic tissue levels of α-pinene. The abundance of plant material and the absence of aggressive predators make it possible that woodrats can rely on this adaptive feeding behavior and easily survive in certain geographic regions [47]. Another important result in the experiments with woodrats was the observation that *Neotoma stephensi* had a high expression of enteric P-gp as compared with the feeding generalist *Neotoma albigula* (Table 2) [48, 49]. This finding underlined the importance of a functional intestinal barrier as the first line of defense against toxic PSM in monogastric species.

## 4 Efflux transporters: Functional elements of the intestinal barrier

For many decades, the epithelial cell layer, closely connected by tight junction proteins, has been described as the barrier system of the intestines, preventing the absorption of many PSMs, as well as biocides and drugs. This assumption was supported by the identification of specific transporters that facilitate the absorption of polar nutrients, such as amino acids and sugars (soluble ligand carriers previously denoted as organic anion transporters, glucose transporters, and others) [50].

**Table 2.** Intestinal efflux transporters expressed at the apical side of enterocytes, their physiological ligands, and examples of indentified PSMs substrates [48, 49, 52, 59]

| Transporter | Tissues (intestines and others) | Physiological substrates | Examples of PSMs known to be substrates |
|---|---|---|---|
| P-gp (MDR1, ABCB1) | Intestines,<br>Blood tissue barriers, brain, choroid plexus, placenta, prostate, ovary, kidneys, liver, lung, skeletal muscle, spleen | Neutral and cationic hydrophobic compounds, amphipathic substances and drugs. | Flavonoids<br>Diterpenes<br>Piperidine alkaloids |
| MRP 2 (ABCC2) | Intestines, brain, placenta, kidneys, liver, lung | Amphipathic organic anions, organic anions, anionic conjugates (glucuronides, sulphates), GSSG, GSH, leukotriene C4) | Flavonoids<br>Isothiocyanates |
| MRP 4 (ABCC4) | Small intestines, brain, testis, kidney, liver, gall bladder, lung, spleen, tonsils, thymus, ovaries, prostate, pancreas, skeletal muscle | Conjugated steroids, bile acids, folic acid, cGMP, cAMP | Flavonoids<br>Isothiocyanates<br>Rotenoids |
| BCRP (ABCG2) | Intestine, breast, ovary, placenta, kidney, liver, lung, heart, spleen, thymus | Amphipathic compounds, organic anions, conjugated organic anions, conjugates (glucuronides, sulphates, glutathione), weak bases | Flavonoids. |

 

Among the many functional proteins and enzymes in the gut wall, a glycoprotein (P-gp) was identified already in 1976, occurring in a distinct pattern in enterocytes of the intestinal wall [51]. As no specific function could be attributed to this protein, it did not get significant attention for more than two decades. This situation changed when the same protein was detected on tumor cells, and particularly on tumor cells of patients that became resistant to various commonly used cytostatic agents. Hence P-gp was described as multi-drug resistance protein (MDR-1). MDR-1 confers resistance to cytostatic agents by pumping these lipophilic compounds out of the tumor cell, thus preventing their interaction with intracellular target sites. Years later and by way of an accident it was found that P-gp (MDR-1) recognizes not only cytostatic drugs, but also a large variety of medicinal products, and even more importantly numerous PSMs [52–54]. This makes P-gp and other efflux transporters that pump their substrates back into the luminal compartment potent functional elements of the intestinal barrier and other biological barriers, such as the blood brain barrier and the placental barrier. It is now well established that MDR-1 is a member of a large family of ATP-dependent transporters, denoted ABC transporters as they share an ATP-binding cassette (http://nutrigne.4t.com/human-abc.htm). ABC transporters play an indispensable role in limiting drug and xenobiotic absorption from the gastrointestinal tract, preventing their distribution into vulnerable tissues [55].

Numerous reports describe the inhibition of Pg-P (MDR1, ABCB1) by PSMs. Nabekura *et al*. investigated the effect of selected flavonoids (quercetin and (−)-epigallocatechin, sesamine (from sesame seeds), matairesinol (from soybeans)), glycerrhetinic acid, and glabridin from liquorice in *in vitro* assays with P-pg over-expressing cells [54]. All selected dietary phytochemicals increased the accumulation of daunorubicin, which was used as a functional marker to demonstrate Pg-P activity. Interestingly, glycerrhetinic acid was found to reverse multi-drug resistance in some cells by modulating ATPase activity. A selective inhibition of P-gp activity has been also described for steroidal saponins from *Paris polyphylla*, of which the rhizome is used widely in traditional medicine for its analgesic, antipyretic, anti-inflammatory, and antitumor properties [56]. Both reports had been driven by the intention to identify compounds that might be useful additives to common anti-cancer therapies with cytostatic agents. However, as herbal remedies are increasingly used as food supplements or health foods in industrialized countries, the P-gp inhibiting effect of PSMs might comprise an unexpected risk, leading to drug–drug interactions. This became obvious when *Hypericum perforatum* (St. John's wort) was widely propagated as natural antidepressant. The crude extract of *Hypericum* contains a complex mixture of hypericin (the major anti-depressant), hyperforin, quercitin, isoquercitin, biflavonoids, naphthoandrones, catechins, and tannins and many other minor constituents. Long-term use of Hypericum formulations is known to interfere with dynamic properties of other antidepressants of the serotonin-reuptake inhibitor group, and kinetic interactions have been described resulting in reduced plasma concentrations of structurally diverse drugs such as for example cyclosporine, digoxin, theophylline, midazolam, indanavir, and saquinavir [57]. Other common herbal remedies known to cause interactions with therapeutic agents are *Allium sativa* (garlic), *Gingko biloba, Panax ginseng, and Silybum marianum* when used in higher concentrations [58, 59]. Undesirable changes in the absorption rate are particularly related to the expression of MDR-1 and CYP3A4, the major sensors of PSMs [60].

In farm animal species, the effects of PSMs on ABC transporters have been studied only to a very limited extent in pigs, poultry, and clinical studies in sheep, despite the fact that exposure to PSMs occurs daily over the entire life span [61–63]. Probably the most prominent example of a compound that is regularly present in animal's diets is pheophorbide A, a breakdown product of chlorophyll. Pheophorbide A has been shown to be a selective BCRP (ABCG2) substrate in all tested mammalian species and as it is expressed in the intestines, can modulate the oral bioavailability of other xenobiotics [64]. BCRP is also present in the mammary gland where it facilitates the excretion of xenobiotics with milk, contributing to body clearance, but also the contamination of milk with drugs and plant metabolites [65]. The latter finding is of importance for the prediction and assessment of undesirable substances in milk for human consumption.

PSMs that are substrates for efflux transporters are often also substrates for drug metabolizing enzymes, particularly CYP450 enzymes. The risk for undesirable effects or intoxications is increased when both, the efflux transporters as well as the predominant CYP450, are inhibited [66].

## 5 Pre-systemic elimination by biotransformation

The important role of hepatic and pre-hepatic biotransformation enzymes limiting systemic exposure to potentially hazardous substances was recognized even before the identification of ABC efflux transporters. The most prominent enzyme system facilitating biotransformation processes is the superfamily of cytochrome P450 enzymes, an ancient enzyme family that can be found in all living organisms [67]. The mammalian enzyme families CYP1 to CYP4 are the most competent in oxidizing and hydrolyzing xenobiotics, including PSMs. The resulting oxidation products are generally more polar, less biologically active, and readily excreted *via* the kidneys. Certain CYP450 isozymes and other Phase I enzymes can also activate PSMs, converting them into electrophilic compounds that may interact with DNA and cause mutagenicity or carcinogenicity [68]. This metabolic activation has first been recognized for the pro-carcinogens benzo(a)pyrene and aflatoxin $B_1$, as

 

their oxidation results in highly reactive epoxides [69, 70]. Within the group of PSMs, the pyrrolizidine alkaloids are a prominent class of multiple compounds that require metabolic activation to form toxic imines [71, 72].

CYP450 enzymes are expressed in all tissues that are potentially exposed to xenobiotics. Recent reviews [73–75] have already summarized the current knowledge regarding the interaction of PSMs with efflux transporters and selected CYP450 enzymes. Typical examples are the induction of CYP3A4 by Hyperforin from St. John's Wort [76], the induction of CYP1A1 and UDP-glucuronosyl transferase (UGT) 1A1 by curcumine, or the differential induction of CYP1A1 by quercetin, whereas CYP1A2 and CYP3A4 are inhibited [73, 77].

While the liver remains the organ with the highest catalytic activity of CYP450 enzymes, significant (and often underestimated) enzyme levels can be found in all cells lining the gastro-intestinal tract [66, 78]. Polymorphisms in individual enzymes can modulate the susceptibility of individuals to certain (classes of) chemicals, a phenomenon that has been intensively studied in relation to drug dosing regimens, and the prevalence of undesirable side effects of common drugs in certain human subpopulations [75]. In animals, specific polymorphisms are less well investigated, but comparative studies in cattle and horses confirm age- and gender-specific expression levels as well as inter-species and inter-breed differences, which makes it difficult to extrapolate data related to the biotransformation capacity between individual animals or animal populations [79–83].

## 6 Transcriptional regulation of efflux transporters and biotransformation enzymes

The transcriptional regulation of biotransformation enzymes remained a matter of controversial debate for many decades, despite the many observations on enzyme induction in response to certain chemicals and the modulation of enzyme activities in the course of systemic infectious diseases. Only within the last decade a specific group of orphan nuclear receptors involved in the regulation of the expression of both biotransformation enzymes and ABC transporters has been elucidated. Major nuclear receptors facilitating constitutive and induced gene expression are the pregnane X receptor (PXR), constitutive androstane receptor (CAR), aromatic hydrocarbon receptor, glucocorticoid receptors, peroxisome proliferator activated receptor, activator protein-1 (AP-1), nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), liver X receptor, and farnesoid X receptor [84, 85]. PXR, CAR, farnesoid X receptor, liver X receptor bind as heterodimers to the retinoid X receptor and then to DNA responsive elements. Upon activation by xenobiotics the ligand–receptor complex is translocated to the nucleus, except for PXR, which is already located in the nucleus. Within the nucleus the complex binds to responsive elements located upstream of the relevant gene in the promoter region and regulates gene expression.

Recent studies have shown that these transcription factors do not only regulate the expression of CYP450 enzymes and some other Phase I drug metabolizing enzymes, but also phase II enzymes and ABC transporters [86]. Typical examples are PXR and CAR, which have a broad and overlapping xenobiotic specificity. Being physiologically involved in the homeostasis of cholesterol and bile acids, they play a pivotal role also in regulating the expression of CYP3A, CYP2B, CYP2C, and the phase II enzymes UGT, sulphotransferases, and glutathione-*S*-transferase [87]. In particular, PXR is recognized as a predominant regulator of CYP3A4, an important enzyme, which is involved in the metabolism of many drugs in humans, both in the intestines as well as in the liver. PXR facilitates also the transcription of MDR-1 (ABCB1) one of the most effective efflux transporters in the gastrointestinal tract as described in Section 4. CYP3A4 and P-glycoprotein have overlapping substrate specificities and similarities in their induction patterns, emphasizing their synergistic activity in contributing to a functional intestinal barrier [88, 89]. Many phytochemicals are able to activate PXR [90] and other transcription factors and hence may alter the levels of expression of biotransformation enzymes and efflux transporters [91]. An alternative pathway is the activation of the transcription factor Nrf2, which is activated by cellular oxidative stress. Nrf2 dimerises with antioxidant responsive element, a specific DNA-promoter-binding region that activates the transcription of a wide array of Phase II enzymes [92]. The direct transcriptional activation of antioxidant responsive element is induced by various PSMs, including diphenols, quinolones, isothiocyanates, and dithiothioles [93]. These detoxification pathways are now considered as a major target for chemoprevention, *i.e.* the blockade of DNA damage by carcinogenic insults.

## 7 Carnivorous species: When plant metabolites become lethal

Glucuronidation is the most prominent pathway for many plant-derived phenols, coumarins, flavonoids, anthraquinones, and steroid(-like) compounds possessing a hydroxyl, amino, carboxyl of sulfhydryl group. Significant glucuronidation activity is found in the small intestines and in hepatocytes, contributing to pre-systemic inactivation and elimination of drugs and toxins. Plant-derived substances such as cruciferous isothiocyanates or piperine can inhibit UGT impairing not only the elimination of these molecules but also affecting the rate of elimination of other endogenous and exogenous compounds [94].

In animals, the extremely low expression of UGT1A in cats is well-known to all feline practitioners. In felidae UGT1A6 is a pseudo-gene that does not allow the transcription of an active enzyme. Subsequently, cats are sensitive to all

 

compounds for which glucuronidation is a major excretion pathway, which is reduced to less than 10% as compared with other animal species. Repetitive exposures (or treatments) result easily in accumulation of the toxin or drug. The most prominent example of this decreased tolerance to drugs in cats is the antiphlogistic acetaminophen, which can induce lethal hepatotoxicity upon repetitive application [94]. Less well defined is the high sensitivity of cats to plant phenols. Common textbooks contain long lists of plants that are considered to be toxic for cats. In all cases these plants contain various phenols and terpenes, which require conjugation to be excreted. In cats these compounds accumulate in the liver, causing mild or progressive liver damage and subsequently loss of appetite, fatty liver syndrome, and in severe cases hepatic encephalopathy.

These molecular mechanisms determining the sensitivity of felidae are of particular relevance to veterinarians and animal poison centers, as it may explain clinical symptoms, following exposure to PSMs and intact plants and point to intervention strategies. At the same time the UGT1A6 pseudogene in felidae is the only gene defect identified in animals that are directly correlated to a high sensitivity towards PSMs.

## 8 Risk assessment of phytochemicals in animal feeds

The risk assessments of phytochemicals in animal feeds needs to cover two different aspects: first, the risk for the target animal for which the feed is intended or which may consume toxic plant materials as part of their normal diet, and second, the risk for the consumer of foods from animal origin, including milk, meat, and eggs. The approach towards this assessment is often entirely different. In the assessment of animal health risks, major toxicological endpoints are organ-specific toxic effects, but also anti-nutritional effects resulting in a reduced feed intake and feed conversion and productivity. As yet, risk assessment of toxic phytochemicals was mainly based on feeding experiments, in which plant materials were mixed in one or more concentrations into an animal diet, and the effects on feed intake and utilization and clinical signs of intoxication were measured. Many of these experiments are of limited value for the risk assessment, as the actual pattern and the concentration of toxic plant metabolites was not measured. Moreover, the rate of absorption of toxic compounds can vary depending on the diet, the actual concentration of one or more plants metabolites in the diet, and the age and breed of the target animal. This emphasizes the demand for mechanism-based studies that provide insights into the absorption, biotransformation, tissue distribution, and routes of excretion of phytochemicals.

In contrast to the assessment of adverse effects of PSMs on animal health and productivity, the evaluation of animal-derived products is driven by the quantities of residues in animal tissues and critical endpoints such as genotoxicity. In many cases, the animal functions as a filter, converting and excreting most of the ingested toxic compounds prior to the use of animal products by the human consumer. This implies that for many phytochemicals the direct exposure *via* the ingestion of plant-derived foods is much higher than that of foods from animal origin. This assumption was confirmed in the recently conducted re-evaluation of undesirable substances in animal feeds, including toxic plant metabolites, as provided by EFSA in the frame of the amendment of Council Directive 2002/32/EC (http://www.efsa.europa.eu). These opinions provide an actual overview about the effects of prominent PSMs that occur in animal feed materials such as cyanogenic compounds, glucosinolates, saponines, tropane alkaloids, pyrrolizidin alkaloids, and individual substances such as ricin, theobromine, or gossypol and animal health. This Directive defines maximum permissible levels for toxic plants and their metabolites in animal feeds, with the objective to protect animal health and productivity. Moreover, an assessment of the potential risks of residues of phytochemicals in animal-derived products is addressed. In this assessment, the excretion of toxic compounds in (dairy) milk, which may result in an undesirable exposure of children, was identified. Children are not only a vulnerable group within the human population, but are also high consumers of milk and dairy products. The recent insights (and testing protocols) into the role of efflux transporters such as BCRP (ABCG2) might serve as an example that mechanistic approaches may contribute significantly to the prediction of undesirable residues of PSMs in animal-derived products.

In conclusion, PSMs are natural ingredients in the diets of herbivorous and omnivorous species. Their occurrence in commercial feed materials cause significant economic losses and limits the use of many protein-rich crops. PSMs impair palatability, weight gain, and performance, and can adversely affect the reproductive capacity and limit the use of otherwise nutritionally valuable plants as animal feeds. Considering the large number of identified PSMs, a systematic analysis of their oral bioavailability and the defense mechanisms that animals have acquired to prevent systemic exposure is essential for the veterinary profession as well as for animal poison centers. The most prominent field for a mechanistic approach in the evaluation of toxic plant metabolites is the risk assessment of feed materials for food producing animals that has to address animal health and productivity as well as the safety of consumers of products from animal origin.

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## 9 References

[1] Feeny, P., Plant appearance and chemical defense. *Recent Adv. Phytochem.* 1976, *10*, 1–40.

 

[2] Bennet, R. N., Wallsgrove, W., Secondary metabolites in plant defense-mechanisms. *New Phytol.* 1994, *127*, 617–633.

[3] Bednarek, P., Osbourn, A., Plant-microbe interactions: chemical diversity in plant defense. *Science* 2009, *324*, 746–748.

[4] Shennan, C., Biotic interactions, ecological knowledge and agriculture. *Phil. Trans. R. Soc.* 2008, *363*, 717–739.

[5] Acamovic, T., Brooker, J. D., Biochemistry of plant secondary metabolites and their effects in animals. *Proc. Nutrition Soc.* 2005, *64*, 402–412.

[6] Allwood, J. W., Ellis, D. I., Goodacre, R., Metabolomic technologies and their application to the study of plants and plant-host interactions. *Physiol. Plant* 2008, *132*, 117–135.

[7] Wink, M., in: Acamovic, T., Stewart, S. C., Pennyscott, T. W. (Eds.), *Poisonous Plants and Related Toxins*, CABI publishing, Wallingford, Oxon 2004.

[8] Haralampidis, K., Trojanowska, M., Osbourn, A. E., Biosynthesis of triterpenoid saponins in plants. *Adv. Biochem. Eng. Biotechnol.* 2002, *75*, 31–49.

[9] Qi, X., Bakht, S., Leggett, M., Hemmings, A. *et al.*, A different function of a member of an ancient and highly conserved cytochrome P450 family: from essential sterols to plant defense. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 18848–18853.

[10] Reed, J. D., Nutritional toxicology of tannins and related polyphenols in forage legumes. *J. Anim. Sci.* 1995, *73*, 1516–1528.

[11] Schardl, C. L., *Epichloe festuca* and related mutualistic symbionts of grasses. *Fungal Genet. Biol.* 2001, *33*, 69–82.

[12] Fink-Gremmels, J., in: Diaz, D. E. (Ed.), *The Mycotoxin Blue Book*, Nottingham University Press, Nottingham, UK 2005, pp 249–268.

[13] O'Brien, M., O'Kiely, P., Forristal, P. D., Fuller, H. T., Fungi isolated from contaminated baled grass silage on farms in the Irish Midlands. *FEMS Microbiol. Lett.* 2005, *247*, 131–135.

[14] Iason, G. R., The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. *Proc. Nutr. Soc.* 2005, *64*, 123–131.

[15] Iason, G. R., Villalba, J. J., Behavioral stretegies of mammalian herbivores against plant secondary metabolites: the avoidance-tolerance continuum. *J. Chem. Ecol.* 2006, *32*, 1115–1132.

[16] Guitart, R., Croubels, S., Caloni, F., Sachana, M. *et al.*, Animal poisoning in Europe Part 1: Farm livestock and poultry. *Vet. J.* 2009. doi:10.1016/j.tvjl.2009.03.002.

[17] Berny, P., Caloni, F., Croubels, S., Sachana, M. *et al.*, Animal poisoning in Europe Part 2: Companion animals. *Vet. J.* 2009. doi:10.1016/j.tvjl.2009.03.034.

[18] Annison, E. F., Bryden, W. L., Perspectives on ruminant nutrition and metabolism. I Metabolism in the Rumen. *Nutr. Res. Rev.* 1998, *11*, 173–198.

[19] Hammond, A. C., Leucaena toxicosis and its control in ruminants. *J. Anim. Sci.* 1995, *73*, 1478–1492.

[20] Allison, M. J., Hammond, A. C., Jones, R. J., Detection of ruminal bacteria that degrade toxic dihydroxypyridine compounds produced from mimosine. *Appl. Environ. Microbiol.* 1990, *56*, 590–594.

[21] Akingbade, A. A., Nsahlai, I. V., Bonsi, M. L., Morris, C. D., du Toit, L. P., Reproductive performance of South African indigenous goats inoculated with DHP-degrading rumen bacteria and maintained on *Leucaena leucocephala*/grass mixture and natural pasture. *Small Rumin. Res.* 2001, *39*, 73–85.

[22] Anderson, R. C., Majak, W., Rassmussen, M. A., Callawat, T. R. *et al.*, Toxicity and metabolism of the conjugates of 3-nitropropanol and 3-nitroproprionic acid in forages poisonous to livestock. *J. Agric. Food Chem.* 2005, *53*, 2344–2350.

[23] Lu, C. D., Jorensen, N. A., Alfalfa saponins affect site and extent of nutrient digestion in ruminants. *J. Nutr.* 1987, *117*, 919–927

[24] Valdez, F. R., Bush, L. J., Goetsch, A. L., Owens, F. N., Effect of steroidal sapogenins on rumen fermentation and on production of lactating dairy cows. *Anim. Feed Sci. Technol.* 1999, *78*, 11–20.

[25] Makkar, H. P. S., Sen, S., Blummel, M., Becker, K., Effects of fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria* and *Acacia auriculoformis* on rumen fermentation. *J. Agric. Food Chem.* 1998, *46*, 4324–4328.

[26] Goel, G., Makkar, H. P. S., Becker, K., Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. *J. Appl. Microbiol.* 2008, *105*, 770–777.

[27] Fahey, J. W., Zalcman, A. T., Talalay, P., The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 2001, *56*, 5–51.

[28] Halkier, B. A., Gershenzon, J., Biology and biochemistry of glucosinolates. *Ann. Rev. Plant Biol.* 2006, *57*, 303–333.

[29] Kliebenstein, D. J., Kroymann, J., Mitchell-Olds, T., The glucosinolate-myrosinase system in an ecological and evolutionary context. *Curr. Opin. Plant Biol.* 2005, *8*, 264–271.

[30] Griffiths, D. W., Birch, A. N. A., Hillmann, J. R., Antinutritional compounds in the Brassicaceae–Analysis, biosynthesis, chemistry and dietary effects. *J. Hort. Sci. Biotech.* 1998, *73*, 1–18.

[31] Tripathi, M. K., Mishra, A. S., Glucosinolates in animal nutrition. *Anim. Feed. Sci. Technol.* 2007, *132*, 1–27.

[32] Waghorn, G. C., McNabb, W. C., Consequences of plant phenolic compounds for productivity and health of ruminants. *Proc. Nutr. Soc.* 2003, *62*, 383–392.

[33] McSweeney, C. S., Palmer, B., McNeill, D. M., Krause, D. O., Microbial interactions with tannins: nutritional consequences for ruminants. *Anim. Feed. Sci. Technol.* 2001, *91*, 83–93.

[34] Aerts, R. J., Barry, T. N., McNabb WC, Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agric. Ecosyst. Environ.* 1999, *75*, 1–12.

[35] Serrano, J., Puupponen-Piia, R., Dauer, A., Aura, A.-M., Saura-Calixto, F., Tannins, current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nutr. Food Res.* 2009, *53*, S310–S329.

[36] Patra, A. P., Saxena, J., Dietary phytochemicals as rumen modifiers: a review on the effect on microbial populations. *Antonie van Leeuwenhoek*, 2009, DOI 10.1007/s10482-009-9346-1.

 

[37] Smith, A. H., Zoetendal, E. G., Mackie, R. I., Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microb. Ecol.* 2005, *50*, 197–205.

[38] Makkar, H. P. S., Blummel, M., Becker, K., In vitro effects and interactions between tannins and saponins and fate of tannins in the rumen. *L. Sci. Food Agric.* 1995, *69*, 841–893.

[39] Newbold, C. J., Hassan, S. M. E., Wang, J., Ortega, M. E., Wallace, R. J., Influence of foliage from African multipurpose tree on activity of rumen protozoa and bacteria. *Br. J. Nutr.* 1997, *78*, 237–249

[40] Chiquette, K., Cheng, K. J., Rode, L. M., Millgan, L. P., Effect of tannin content on two isosynthetic strains of birdsfoot trefoil (*Lotus cornuculatus*) of feed digestibility and rumen fluid composition in sheep, *Can. J. Anim. Sci.* 1989, *69*, 1031–1039.

[41] Barry, T. N., McNabb, W. C., The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *Br. J. Nutr.* 1999, *81*, 263–272.

[42] Wallace, R. J., Antimicrobial properties of plant secondary metabolites. *Proc. Nutr. Soc.* 2003, *63*, 621–629.

[43] Greathead, H., Plants and plant extracts for improving animal productivity. *Proc. Nutr. Soc.* 2003, *62*, 279–290.

[44] Busquet, M., Calsamiglia, S., Ferret, A., Kamel, C., Plant extracts affect in vitro rumen microbial fermentation. *J. Dairy Sci.* 2006, *89*, 761–771.

[45] Rochfort, S., Parker, A. J., Dunshea, F. R., Plant bioactives for ruminant health and productivity. *Phytochemistry* 2008, *69*, 299–322.

[46] Freeland, W. J., in: Palo, R., Robbins, C. T. (Eds.), *Plant Defences Against Mammalian Herbivory*. CRC, Boca Raton, USA 1991, pp. 61–82.

[47] Dearing, M. D., Mangione, A. M., Karasov, W. H., Diet breath of mammalian herbivores: nutrient versus detoxification constrains. *Oecologica* 2000, *123*, 397–405.

[48] Green, A. K., Haley, S. L., Dearing, M. D., Barnes, D. M. *et al.*, Intestinal capacity of P-glycoprotein is higher in the juniper specialist, *Neotoma stephensi*, than the sympatric generalist, *Neotoma albigula*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 2004, *139*, 325–333.

[49] McLean, S., Duncan, A. J., Pharmacological perspectives on the detoxification of secondary plant metabolites: implications for ingestive behaviour of herbivores. *J. Chem. Ecol.* 2006, *32*, 1213–1228.

[50] Palacín, M., Estévez, R., Bertran, J., Zorzano, A., Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev.* 1998, *78*, 969–1054.

[51] Juliano, R. L., Ling, V., A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta* 1976, *455*, 152–162.

[52] Dietrich, C.G., Geier, A., Oude Elferink, R.P., ABC of oral bioavailability: transporters as gatekeepers in the gut. *Gut* 2003, *52*, 1788–1795.

[53] Takano, M., Yumoto, R., Murakami, T., Expression and function of efflux drug transporters in the intestine. *Pharmacol. Ther.* 2006, *109*, 137–161.

[54] Nabekura, T., Yamaki, T., Ueno, K., Kitagawa, S., Inhibition of P-glycoprotein and multidrug resistance protein 1 by dietary phytochemicals. *Cancer Chemother. Pharmacol.* 2008, *62*, 867–873.

[55] Oostendorp, R. L., Beijnen, J. H., Schellens, J. H., The biological and clinical role of drug transporters at the intestinal barrier. *Cancer Treat. Rev.* 2009, *35*, 137–147.

[56] Nguyen, V. T., Darbour, N., Bayet, C., Doreau, A. *et al.*, Selective modulation of P-glycoprotein activity by steroidal saponines from *Paris polyphylla*. *Fitoterapia* 2009, *80*, 39–42.

[57] Zhou, S., Chan, E., Pan, S.Q., Huang, M. *et al.*, Pharmacokinetic interactions of drugs with St John's wort. *Psychopharmacology* 2004, *18*, 262–276.

[58] Wu, C. P., Calcagno, A. M., Hladky, S. B., Ambudkar, S. V., Barrand, M. A., Modulatory effects of plant phenols on human multidrug-resistance proteins 1, 4 and 5 (ABCC1, 4 and 5). *FEBS Lett.* 2005, *272*, 4725–4740.

[59] Telang, U., Ji, Y., Morris, M. E., ABC transporters and isothiocyanates: potential for pharmacokinetic diet-drug interactions. *Biopharm. Drug Dispos.* 2009. DOI: 10.1002/bdd.668.

[60] Pal, D., Mitra, A. K., MDR- and CYP3A4-mediated drug-herbal interactions. *Life Sci.* 2006, *78*, 2131–2145.

[61] Brand, W., Schutte, M. E., Williamson, G., van Zanden, J. J. *et al.*, Flavonoid-mediated inhibition of intestinal ABC transporters may affect the oral bioavailability of drugs, food-borne toxic compounds and bioactive ingredients. *Biomed. Pharmacother.* 2006, *60*, 508–519.

[62] Schrickx, J. A., Fink-Gremmels, J., Implications of ABC transporters on the disposition of typical veterinary medicinal products. *Eur. J. Pharmacol.* 2008, *585*, 510–519.

[63] Haritova, A. M., Schrickx, J. A., Lashev, L. D., Fink-Gremmels, J., Expression of MDR1, MRP2 and BCRP mRNA in tissues of turkeys. *J. Vet. Pharmacol. Ther.* 2008, *31*, 378–385.

[64] Robey, R. W., Steadman, K., Polgar, O., Morisaki, K. *et al.*, Pheophorbide is a specific probe for ABCG2 function and inhibition. *Cancer Res.* 2004, *64*, 1242–1246.

[65] Alvarez, A.I., Real, R., Pérez, M., Mendoza, G. *et al.*, Modulation of the activity of ABC transporters (P-glycoprotein, MRP2, BCRP) by flavonoids and drug response. *J. Pharm. Sci.* 2009. DOI 10.1002/jps.21851.

[66] Sergent, T., Ribonnet, J., Kolosova, A., Garsou, S. *et al.*, Molecular and cellular effects of food contaminants and plant components and their plausible interactions at the intestinal level. *Food. Chem. Toxicol.* 2008, *46*, 813–841.

[67] Nebert, D. W., Russell, D. W., Clinical importance of the cytochromes P450. *Lancet* 2002, *360*, 1155–1162.

[68] Wogan, G. N., Hecht, S. S., Felton, J. S., Conney, A. H. *et al.*, Environmental and chemical carcinogenesis. *Semin. Cancer Biol.* 2004, *14*, 473–486.

[69] Gelboin, H.V., Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev.* 1980, *60*, 1107–1166.

[70] Guengerich, F. P., Johnson, W. W., Ueng, Y. F., Yamazaki, H., Shimada, T., Involvement of cytochrome P450, glutathione S-transferase, and epoxide hydrolase in the

 

metabolism of aflatoxin B1 and relevance to risk of human liver cancer. *Environ. Health Perspect.* 1996. *104*, 557–562.

[71] Huan, J. Y., Miranda, C. L., Buhler, D. R., Cheeke, P. R., Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. *Toxicol. Lett.* 1998, *99*, 127–137.

[72] Fu, P. P., Xia, Q., Lin, G., Chou, M. W., Pyrrolizidine alkaloids-genotoxicity, metabolism, enzymes, metabolic activation and mechanisms. *Drug Met. Rev.* 2004, *36*, 1–55.

[73] Zhou, S., Gao, Y., Jiang, W., Huang, M. *et al.*, Interactions of herbs with cytochrome P450. *Drug Metab. Rev.* 2003, *35*, 35–98.

[74] Moon, Y. J., Wang, X., Morris, M. E., Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. *Toxicol. in Vitro* 2006, *20*, 187–210.

[75] Bosch, T. M., Meijerman, I., Beijnen, J. H., Schellens, J. H., Genetic polymorphisms of drug-metabolising enzymes and drug transporters in the chemotherapeutic treatment of cancer. *Clin. Pharmacokinet.* 2006, *45*, 253–285.

[76] Komoroski, B. J., Zhang, S., Cai, H., Hutzler, J. M. *et al.*, Induction and inhibition of cytochromes P450 by the St John's wort constituent hyperforin in human hepatocyte cultures. *Drug Metab. Dispos.* 2004, *32*, 512–518.

[77] Gross-Steinmeyer, K., Spepleton, P. L., Lis, F., Tracy, J. H. *et al.*, Phytochemical-induced changes in gene expression of carcinogen-metabolizing enzymes in cultures human primary hepatocytes. *Xenobiotica* 2004, *34*, 619–623.

[78] Pavek, P., Dvorak, Z., Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. *Curr. Drug Metab.* 2008, *9*, 129–143.

[79] Dacasto, M., Eeckhoutte, C., Francesca, C., Dupuy, J. *et al.*, Effect of breed and gender on bovine liver cytochrome P450 3A (CYP3A) expression and inter-species comparison with other domestic ruminants. *Vet. Res.* 2005, *36*, 179–190.

[80] Gusson, F., Carletti, M., Albo, A. G., Dacasto, M., Nebbia, C., Comparison of hydrolytic and conjugative biotransformation pathways in horse, cattle, pig, broiler chick, rabbit and rat liver subcellullar fractions. *Vet. Res. Commun.* 2006, *30*, 271–283.

[81] Nebbia, C., Biotransformation enzymes as determinants of xenobiotic toxicity in domestic animals. *Vet. J.* 2001, *161*, 238–252.

[82] Ioannides, C., Cytochrome p450 expression in the liver of food-producing animals. *Curr. Drug. Metab.* 2006, *7*, 335–348.

[83] Fink-Gremmels, J., Implications of hepatic cytochrome P450-related biotransformation processes in veterinary sciences. *Eur. J. Pharmacol.* 2008, *585*, 502–509.

[84] Wang, H., LeCluyse, E. L., Role of orphan nuclear receptors in the regulation of drug-metabolising enzymes. *Clin. Pharmacokinet.* 2003, *42*, 1331–1357.

[85] Kliewer, S. A., Goodwin, B., Willson, T. M., The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr. Rev.* 2002, *23*, 687–702.

[86] Xu, C., Li, C. Y., Kong, A. N., Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch. Pharm. Res.* 2005, *28*, 249–268.

[87] Handschin, C., Meyer, U. A., Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. *Arch. Biochem. Biophys.* 2005, *433*, 387–396.

[88] Lin, J. H., Yamazaki, M., Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin. Pharmacokinet.* 2003, *42*, 59–98.

[89] Sorensen, J. S., Dearing, M. D., Efflux transporters as a novel herbivore countermechanism to plant chemical defenses. *J. Chem. Ecol.* 2006, *32*, 1181–1196.

[90] Satsu, H., Hiura, Y., Mochizuki, K., Hamada, M., Shimizu, M., Activation of pregnane X receptor and induction of MDR1 by dietary phytochemicals. *J. Agric. Food Chem.* 2008, *56*, 5366–5373.

[91] Sergent, T., Ribonnet, L., Kolosova, A., Garsou, S. *et al.*, Molecular and cellular effects of food contaminants and secondary plant components and their plausible interactions at the intestinal level. *Food Chem. Toxicol.* 2008, *46*, 813–841.

[92] Lee, J. S., Surh, Y. J., Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett.* 2005, *224*, 171–184.

[93] Hayes, J. D., Kelleher, M. O., Eggleston, I. M., The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *Eur. J. Nutr.* 2008, *47*, 73–88.

[94] Court, M. H., Greenblatt, D. J., Molecular genetic basis for deficient acetaminophen glucuronidation by cats: UGT1A6 is a pseudogene, and evidence for reduced diversity of expressed hepatic UGT1A isoforms. *Pharmacogenetics* 2000, *10*, 355–369.

 