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MODERN NOTION OF BIOTRANSFORMATION ALYKOTOXINS

Despite the unfavourable influence of mycotoxins on human and animal health and few toxicological aspects that have been documented, about these biologically active substances has not been explored. Aiming ai more knowledge and a better understanding of the effects and mechanism of mycotoxin action in mammals would provide the basics for developing strategies to restrain different mycotoxicoses. One of the processes not fully understood is biotransformation, to which mycotoxins are subjected the animal organism. Biotransformation is the conversion of mycotoxins to non-toxic metabolites and occurs mostly in the intestinal mucosal membrane and liver, although other tissues and systems also take part in this process. Mycotoxin biotransformation reactions can be considered bioinactivation or detoxication, but mycotoxin biotransformation processes could also result in products more toxic than the mycotoxin. It can be concluded from research studies that our knowledge of mycotoxin. biotransformation is scarce.

Key words: *biotransformation, aflatoxin B1, ochratoxin A, deoxynivalenol, fumonisins, zearalenon*

Introduction

All organisms are constantly exposed to mycotoxins the secondary products of metabolic processes in moulds.The rate of mycotoxin removal is often determined by the method of biotransformation for substances chemically soluble in water, which are then enzymatically converted in the liver or other tissues for removal (detoxication). Many biotransformation reactions can be considered as mycotoxin bioinactivation or detoxication. Bioinactivation should be understood as lowering the toxic properties of a molecule; its *status presens* is of the processed form and the toxic properties are reduced or gone. However, biotransformation processes may result in products more toxic than the original mycotoxin. These reactions are normally called bioactivation reactions [1,2] .

Biotransformation is the conversion of toxic substances to non-toxic metabolites during different phase I and phase II biochemical processes, which are the transformation into more hydrophilic substances. During the first phase of detoxication, as the result of the presence of a mycotoxin. higher enzymatic, activity causes oxidation, reduction, and or hydrolysis reactions that expose functional hydroxyl, carboxyl or amine groups [3]. The structure ol the my cotoxin decides which reaction takes place. The enzymes are responsible for initiating the mycotoxin biotransformation processes. In most cases the biotran. sformation process allows substances created during phase 1 to enter conjugation processes, which are the

burden of phase II. In some cases different substances may be eliminated directly after phase I reactions.

The enzymatic system of phase I includes several isoenzymes, of which a few hundred have been identified that show the affinity for different substrates.There are a few types of enzymes that take part in detoxication, but most often they are cytochromes from the P450 superfamily (CYPs) [4]. These cytochromes are responsible for the me-tabolism of most mycotoxins and have a very widerange of substrate specificity. In mammals, CYPs arepresent in the endoplasmic reticulum and mitochondria of most cell [4].The enzymes are most often found in the liver, but increase CYP activity is also found in the intestinal wall, kidneys. lungs, and brain. [2].

The CYP enzymes use oxygen and NADH cofactor to add the hydroxyl group. As a result, new com-pounds arise in phase I that can be more toxic than the original particles. In such a situation, if the newparticles are not included in further biotransformation processes, they can damage proteins, RNA, and DNAin the cell [5]. It has been shown that there is a correlation between phase I induction by CYP activation and/or decreased activity in phase II,which favours neoplastic. [6].

In most cases, phase II reactions results in the decreased biological activity of the original particles.Metabolic conjugation with endogenous hydrophilic substances results in compounds with enough hydrophilic properties to be quickly eliminated. These conjugation reactions may occur with many substan-ces and engage many prosthetic group derivatives(cofactors) that take part in metabolic processes, suchas glucuronic acid, sulphates, glycine, or glutathione.Conjugation during phase 11 is the coupling of intermediate metabolic products with polar groups fromone of the cofactors [2].

To summarize briefly, it can be stated that the above mentioned CYP enzymes probably oxidise different mycotoxins. processing them into particles thatconjugate with polarised cofactors during phase II of the detoxication process. This process is important be-cause some products formed during the biotransformation are more toxic than the original substances,and this process plays a significant role in modellingor increasing the toxic properties of mycotoxins. The products formed, if not immediately transformed byone or more enzymes in phase II, can carry out toxicactions on liver tissue, or the whole organism if they leave hepatic cells [6] . The results of toxicity may be local or systemic and arise from oneor many reactions [1], for example, the covalent binding of reagent metabolites, such as the crossjunction of structural proteins and changes i the spotial structure of receptors, membrane pumps, transport proteins, enzymes, or protein hormones; the phospholipids of cell membranes that bind reactive intermediate substances to facilitate the solubility of xenobiotics in fats and initiate the formation of peroxide groups by oxidation; and amino acids, such as irre-versible DNA damage and cancer genesis [6]. Another example is the development or intensification of oxidative stress; CYPs are an important element in xenobiotic; especially hy-drophobic metabolism. Metabolic products are usually more hydrophilic than the substrates, which favours their further metabolism and excretion. The metabolic products also take part in fatty acid and eicosanoid metabolism; after biotransformation, xenobiotics undergo electrophilic reactions that are quenched by cellular reductive equivalents, such as superoxide dismutase, catalase, or glutathione peroxidase. These reactive forms of oxygen cause oxidative stress. [1]. [6].

From the clinical point of view, during phase I of detoxication, unknown nutritional support is demanded to maintain full activity. This demand is important because, in the case of nutrient deficiency, phase I detoxication increases the activity of the mycotoxin in the animal body as a result of the deposition of different mycotoxins and or xenobiotics being released from fat or nonfat tissues, which causes a high toxic load in the organism. Therefore, in the case of nutrient deficiency in the organism, detoxication processes result in the intensification of different disease states or the presence of clinical signs before reaching the subclinical state. For the effective secretion of mycotoxins, during phase I one should increase the antioxidants in feed and during phase-Ii one should support the animals with a specific diet that activates liver action. [4].

The above mentioned phases have to be proportionally active for an effective course of the detoxication process. The steroids, fatty acids, and other endogenous molecules released during intercellular communication also have a great influence on the variability of detoxication processes in different organisms. Therefore, maintaining the balance between phase 1 and II activity is very important. During the ongoing detoxication process, the degree of tissue damage depends on the strength of the oxidative stress caused by free radicals and oxidants, which occurs when these active intermediate substances are immediately and effectively included in the phase II reaction cycle. If, for some reason, the phase reactions are inhibited or the activity of phase I processes is increased without an increase in phase II activity, the correlation between the processes is disabled, in other words, the balance of the detoxication process is disturbed [7]. Furthermore, phase II needs cofactors to be present'and energetic supplements from source materials, like ATP. [3].

In the detoxication process, the gastrointestinal tract is a physical barrier for exogenous factors, but it also influences the process in other ways.[7]. Microorganisms of the mucosal membranecan produce substances that activate or inhibit detoxi-cation processes. Moreover, as a result of liver-intes-tine recirculation, mucosal membrane microflora canalso partially remove conjugates produced in the liver.

On the other hand, glucoronides can be removed andmycotoxins returned to their original state to circulate and increase the toxic load of the organism.

Detoxifying enzymes, such as the CYP3A4 isoenzyme, and the antiporter activity of proteins in en-terocytes are found in high concentrations in the apical portion of intestinal villi. [8]. During the initiation of the mycotoxin metab-olism process in the gastrointestinal tract, the full in-tegrity of the intestinal mucosal membrane should be present. The impairment of the mucosal membrane and exposure of the intestinal villi facilitates mycotoxin passage to the circulation, skipping the firstphase of detoxication. The primary condition for decreasing the toxic burden is maintenance of the bestfunctional state of the mucosal membrane.

In the last few years, it has been suggested moreoften that the antiporter activity of proteins in enterocytes, such as P glycoprotein or a multidrug resis-tance protein, is a third phase of the detoxication system. [1,2,8 - 10]. This antiporter activity is very important in the firstpart of the metabolism of drugs and mycotoxins. Theprocess carried out by antiporter proteins depends onthe presence of energetic compounds that influenceion pump activity, which ejects mycotoxins out of thecell, resulting in a reduction in the concentration of these substances in the intracellular space. [11] . Many enzymes taking part in detoxication are located near the cell wall and, in the

situation where the mycotoxin or its metabolite is not being biotransformed from the beginning, they are taken upby the cell into the cytosol. Active ion pumps eject themycotoxin out of the cell, and it goes back to theintestinal lumen where it is again taken up by the cellsin a recirculation process that allows the cell to me-tabolize this substance again before it passes deep intothe cytosol, where it could do more damage. The activity of antiporter proteins in the intestines dependson the presence of intestinal enzymes CYP3A4 andphase 1 isoenzyme, which plays a main role in detoxication. [2].

In the last few years, scientific studies have focused on the mycotoxins that cause disease in the human population or worsen the health status of farm or companion animals. [12] . These mycotoxins are most often aflatoxin B_h ochratoxin A, tricothecenes (toxin T-2, deoxynivalenol, and diacetpxyscirpenol), zearalenon, and fumonisins. All of these mycotoxins have been described as structurally unique secondary metabolites of moulds that act in incomparable ways. Aflatoxin B, for instance, has to be biologically inactivated from its initial form to a mutagenic or carcinogenic metabolite. Tricothecenes are commonly known as immunosuppressive factors impairing phagocytic activity, decreasing the IgG and IgM concentration, and causing slower immune reactions. Both T-2 toxin and deoxynivalenol cause the inhibition of protein and DNA synthesis. Zearalenon is commonly known as, a factor that causes impaired endocrine system activity as a result of binding with estrogen receptors. Fumonisins cause the inhibition of sphinganin -N-acetyl transferase activity and ceramide synthesis, which causes an increase in the intracellular concentration of sphinganine substrate [13] .

Despite the growing understanding of the mycotoxin problem, they still influence economy throughout the world. Mycotoxins influence the health and life of humans and animals, animal production, crop yield and feed quality, health care, and veterinary care costs. The key to limiting the influence of mycotoxins in different animal species and humans will be the understanding of the metabolic processes and mycotoxin biotransformation in the organism [1].

Aflotoxins

Most toxicological research concerning biotransformation has been done on aflatoxins. There is great discrepancy between species and, in many cases, between different animals, resulting in varying mycotoxin metabolism. [14] . The pure form of B_1, aflatoxin is not mutagenic[15] . and its biotransformation in the tissues of mammals takes place in the presence of microsomal CYP enzymes that exhibit monooxygenase activity. [4]. These enzymes and their subfamilies are found at varying concentrations in many tissues in many animal species, but the highest concentration is in the liver. We currently know four stages of aflatoxin B, biotransformation: (i) O-deal- kylation to P_1 aflatoxin, (ii) ketoreduction to aflatoxicol processes, (iii) epoxidation processes to B_1-8,9-epoxide aflatoxin, which shows strong toxicity and is mutagenic and carcinogenic, and (iv) hydroxylation to M_1 aflatoxin, which also has a strong toxicity, or aflatoxin P_1, aflatoxin Q_1 or aflatoxin $B_{2a:}$ which are all relatively nontoxic. Numerous forms of CYP are capable of biotransformatioft, which depends on the animal species. Generally, P450 enzymes of the 1A, 2B, 2C, and 3A subfamilies are present during B_1-8,9-epoxide aflatoxin activation. The CYP1A2 isoform, which is induced by polycyclic aromatic hydrocarbons, is primarily important in human tissues. The detoxication of B1-8,9-epoxide aflatoxin and M_1 aflatoxin in mammalian tissues takes place as the result of a reaction with

glutathione (GSH), catalyzedby S-glutathione transferase (GST), which is characteristic for phase 11 of biotransformation. [16].

The efficiency of the biotransformation process, both activation and detoxication, in different animalspecies determines the toxicity of aflatoxins. The B_1 aflatoxin activation processes have been described as very efficient in the presence of mixed oxidases originating from the nose and larynx mucus of swine.

However, swine studies performed in regions where aflatoxins contaminate feed at levels that are detectedin the Irver, respiratory system cancers are more common than liver cancer in piglets fed aflatoxins. In other studies [24], cow hepatocytes metabolized B_1, aflatoxinmostly to M_1 aflatoxin, but also epoxidated B_1, aflatoxin B_1 aflatoxin dihydrodiol, and B_1 aflatoxinconjugated with GSH. In hepatic cell cultures noaflatoxicol was found; however, it was previouslyfound in cow blood plasma, erythrocytes, and milk,which was probably a result of mycotoxin metabolismby rumen flora.

Only 0,5 to *5*% of B_1, aflatoxin taken up in thecows' feed can be transformed to Mi aflatoxin, whichis present in milk. Aflatoxin B, biotransformation iii the hepatic cells of cows and the relative M, aflatoxinconcentration in milk depend on many factors, includ-ing milk yield, the mixed function of microsomaloxidase, and bacteria causing mastitis in the udder. [17].

It has been show-п that aflatoxin detoxication inruminates can be made more efficient by changing -jtheir diet, particularly to another protein source, sueп2I<as fish meal, or including amino acid supplementa-tion, which supports metabolic processes.by providingmethionine. [18].

Deoxynivalenol

Compared to aflatoxins, there has been much less work done concerning the biotransformation of tricothecenes. All animal species are sensitive to thepresence of deoxynivalenol (DON) in feed. The degree of sensitivity is as follows: swine > mice > rats> poultry = ruminants. These differences in sensitivityare interpreted as being due to different courses'of absorption, metabolism, distribution, and eliminationof the mycotoxin [19, 20, 21]. Short-term intoxication ofmonogastric mammals by this mycotoxin causes theactivation of enzymes taking part in both phases of biotransformation, accompanied by no differences in the expression of CYP, which reveals no differences in DON cytotoxicity [22].This mycotox conjugates with glucoronic acid in the liver after previ ous conversion in the animal tissues. [23]. The main metabolite of DON, de-epoxy-DON (DOM – 1), is present in the urine and faeces of examined animals (Fig. 1). This metabolite is formed mainly due to intestinal or rumen bacterial flora and not in the internal organ [24]. In the case of humans, which depend on gastrointestina tractfficiency durin gthemetabolism of 3-acetyldeoxynivalenol and nivalenol, no DOM-1 has been found in faeces [25]. Differences in the metabolic course of tricothecenes in the gastrointestinal tract in different animal speciesreveal the importance of their toxicity.

In swine, the adsorption of DON is fast. The highest level of mycotoxin found in blood plasma occursafter 30 minutes of oral administration. The majorityof this mycotoxin enters the organism via the oralroute. The absorption process takes part mainly in theposterior small intestine. Some authors have sugges-ted that de-epoxidation takes place mainly in the largeintestine (Fig. 1); therefore, this process is not import-ant for detoxication in swine. The detoxifying factorfor the absorption or

metabolism of DON in the gastrointestinal tract of swine should be effective in the physiological states of the stomach and duodenum at a very specific time [26].

Lower than in swine, the sensitivity of chickens to the presence of DON in feed comes from a low degree of absorption into the blood plasma and tissues, as well as the fast clearance of birds. A similar situation occurs in turkey [27]. The intestinal flora of poultry metabolizes DON to DOM-1. Small amounts of this mycotoxin is deposited in the tissues and eggs [23].

Ruminants are also resistant to the presence of DON in feed. After, oral administration, the highest concentration of DON is found 4 hours later in a conjugated form of p-glucuronate. Both free and conjugated DON is detected in milk and is excreted from the cow in very low concentrations (Fink-Gremmels 2008). A similar situation occurs in sheep.

To summarise, in feed, bovine and poultry tolerate 20 ppm DON and pigs exhibit disease signs after a dose lower than 1-2 ppm. The difference in tolerance is caused by a different course of toxokinetic processes, especially biotransformation, in the different animal species. The bacterial flora of the gastrointestinal tract, especially rumen, plays a deciding role in the DON detoxication process. It appears that DON is metabolized mainly by gastrointestinal tract microorganisms in all species of animals and humans, and it is not deposited in the tissues. Therefore, DON residues in food of animal origin are not serious from a food safety point of view [23].

Ochratoxin

Ochratoxin (OT)A biotransformation is not fully understood and data concerning its metabolism are controversial. Few metabolites have been characterised *in vitro* or *in vivo*, and those that have been are still being explored. The participation of OTA metabolites in the toxicity process is still ambiguous.

The main route of OTA metabolic processes is hydrolysis, and the products are far less toxic. In most animals, detoxication occurs mainly thanks to caecal microflora[18,28,29]. Anaerobic microorganisms, particularly bacteria in the colon and caecum, are responsible for hydrolytic processes, although the enzymes necessary for these reactions are not produced by these fragments of the gastrointestinal tract but probably by microorganisms living in them. Much higher resistance to the toxic effects of OTA has been documented in ruminants, which is attributed to the detoxication abilities of rumen microflora. It has been shown in previous studies that, in the liquid content of rumen, OTA breaks down to OTa. This hydrolysis is also influenced by rumen protozoa. It has been stated that the activity level of rumen during OTA hydrolysis depends on the animals' diet, and an increased concentration of starch in the diet causes increased numbers of proto, zoa in the rumen [29].

In a small percentage of animals, absorbed OTA is hydrolysed to OTa. During the initial phases of detoxication, hydroochratoxin A is produced in the liver. The OTA enters the liver through the portal vein. Initially, detoxication takes place in hepatocytes (Fig. 2). During this process, OTA is metabolized in the hepatic microsomes and two epimers arise. 4(R)-OH OTA and 4(S)-OH OTA. The 4(R)-OH OTA is mainly produced by the microsomal system of human and rat livers, and 4(S)-OH OTA is formed by the system in the swine liver. The low toxicity of these metabolites towards mammals can be attributed to their easy elimination, similar to OTA. There is no data concerning the toxicity of 4(S)-OH OTA [2].

The number of OTA biotransformation products does not explain the wide range of toxic effects and nephrotoxic and carcinogenic activity. The bioactivation

reactions are still not fully understood for this mycotoxin. However, we currently do know for sure that the enzymes taking part in OTA biotransformation are a CYP isoform, prostaglandin synthase (POSH), and lipoxygenase (FOX).

Generally, phase 1 of mycotoxin biotransformation is catalysed by the CYP system. These enzymes are present mainly in the liver but also in other tissues, such as the intestinal mucosal membrane, lungs, nose mucosal membrane, skin cells, kidneys, testicles, ovaries, and placenta. In eukaryotic cells, P450 isoforms engaged in endogenous compound biosynthesis are present in the smooth endoplasmic reticulum. Data concerning CYP engagement in OTA metabolism processes are still contradictory [29].

.Generally, during phase II of biotransformation, mycotoxins mainly conjugate with glucoronic or sul phate groups or glutathione. These processes occur mainly in the liver, which is a very important organ engaged in xenobiotic glucuronidation processes. At the cellular level, UDP-glucuronosyltransfera.se (UGT) is a microsomal membrane enzyme neighbouring CYP. Endogenous glucoronic acid can be conjugated with the phenol or carboxyl group of OTA. Conjugated glucuronides may be excreted with bile and then hydrolysed to aglicans by the beta-glucuronidase of intestinal microflora. The released mycotoxin may by reversibly absorbed (reabsorption) and the cycle repeated. However, as a result of different extrapolations concerning OTA conjugation with glucorouians or sulphates *in vitro* and *in vivo*, the problem is still not solved. Equally unknown is the eventual OTA conjugation with glutathione, because some authors suggest that the quantitative participation of conjugated OTA with glutathione during the biotransformation process is not more than 1%; thus, more difficult to detect analytically [29]. It appears that, apart from some acquired knowledge, OTA is still a mysterious mycotoxin .

Fumonisins

Fumonisin B, (FBj) is one of most popular mycotoxins and causes many health problems in humans and animals [30]. This mycotoxin behaves differently in the gastrointestinal tract than other mould metabolites after feed intake. One of the elements of the dissimilarity is the low biological availability of FB, compared to ochratoxin or patulin [31]. Coupled with no changes in the integrity of the mucosal membrane of the gastrointestinal tract, this mycotoxin cannot be a dangerous substance for the organism in which it is present. As a result, there are no factors that cause the arrest of phase I of biotransformation. Until recently, there was no 'Correlation between fumonisin activity and metabolic enzyme activation for the hydrolysis occurring during biotransformation (phase I or/and II) [32]. The phenomenon of potentiation, hyper additive synergism, which is the amplification of one of the mycotoxin actions by another mycotoxin, is probably how the fumonisin mycotoxins act [33]. Hypothetically, one can assume that another mycotoxin or mycotoxins, as in mixed mycotoxicoses [34], would have to break the integrity of the mucosal membrane of the gastrointestinal tract and create an entrance for FB, as two dosages of two mycotoxins taken up in the feed over a long period of time. [33 - 37].[33].

After breaking through the intestinal barrier, fumonisins, which have a first class amine group at C2, competitively inhibit ceramide synthase, disrupt the *de novo* biosynthesis of cerarnides, and interrupt sphingolipid metabolism [38].

As an immediate effect of ceramide synthase inhibition. the accumulation of sphingoid base enzymes, which bind sphinganine.(Sa), decreases the presences of

sphingosine (So) in the tissues, serum, and urine. Sphingoid base accumulation and a simultaneous increase in the Sa:So ratio in tissues confifms the presence of fumonisin, which has been documented in different mammals, birds, and fish, and is used as a biological marker of fumonisinotoxicosis [38].

Of three known FB, metabolites, only aminopentol (HFB_1) [39] is totally hydrolysed. The HFB_1 is created when strong alkaline substances act on crop corns (nixtamalisation). Aminopentol is more polar than HFB_1 and higher absorption and toxicity has been shown after oral administration. The target of the toxic activity of FB, is not the intestinal lumen; the intestine is exposed to HFB, before passing the intestinal barrier and there is a hypothetical concern regarding protein antiportcr activity in en- terocytes, for instance P glycoproteins that would result in the return of the toxic HFB, metabolite to the intestinal lumen. Moreover, the kinetically Saturated P glycoprotein has many ligands with high specificity and is present with other substrates in the intestinal lumen and/or inhibition factors, which can cause an increase in the biological availability of HFB, [32].

Zearalenon

Zearalenon (ZEA) is a non-steroid mycotoxin with estrogen activity and a specific hormone regulating the sexual reproduction of *Fusarium* (sexual stage *Gibberella zeae*). The identification of factors participating in ZEA biotransformation is necessary to understand the distribution of mycotoxin in the organism and metabolism processes influencing the modification of its physicochemical properties [40].

Depending on the biochemical efficiency, detoxi-cation can take different courses. During mycotoxinintoxication at levels not requiring a higher activity ofphase 1 enzymes, the total elimination of the toxicfactor, ZEA. has been observed. In the situation ofphase I enzyme activity being too low for the amountof ZEA taken, there is an imbalance between phase1 and 11, and metabolites type and (3-zearalenol(ZOL) enter the organism; this can result in the altered activity of enzymes taking part in steroidogenesisor hormonal regulation on the prereceptor level.

Fuzariotoxins, including ZEA. metabolism takesplace mainly in the liver, to a lower degree in thegastrointestinal tract, probably in the rumen of ruminants, and in the final segment of alimentary tract with bacterial flora [41]. These changes occur before absorption. Almost 90%of ZEA is reduced to α-ZOL and (β-ZQL. Danicke al. (2002) showed that, in ruminants, ZEA and metabolites are found in bile as 68% β-ZOL, α -ZOL. and 8% ZEA.

There is more information available about mycotoxin biotransformation in different species by intestinal microorganisms than other processes. These are mostly studies describing the metabolism of DON in swine [42 - 44]. or fuzariotoxins (Kimura et al. 2006). In the case ol ZEA, it has been suggested that intestinal microorganisms hydrolyse the substance only to α -ZOL [45].

The biotransformation of ZEA to a-ZOL and (3-ZOL) is caused by ketone group reduction at the position [45]. This reaction is very similar to processes taking placc during the metabolism of steroids catalysed by hydroxysteroid de hydrogenase (HSD) [47].

Studied the result of ZEA [48] biotransformation in granular cells with, or without, endogenous substrates for 3α- and 3β-HSD (Fig. 4.). Previous studies showed that 3a-HSD is engaged in ZEIA reduction to a-ZOL. It should be remembered that more than one metabolite often arises from the original substrate,

which can be conjugated with other substances and be inactivated [49] or remain a free active compound. The results of these studies documented that not only do swine granular cells transform ZEA to α -ZQL and β-ZOL but there are significant changes in the enzymatic biotransformation speed of both metabolites. Similarly, biotransformation takes place in the liver [35] and the main metabolite of ZEA in swine granular cells (Malekinejad et al) is α - ZOL. Endogenous hormones, such as 5α-DHT or progesterone. are the physiological substrates for 3 α -HSD. Pregnenolone is the most important product and substrate for 3β-HSD. All three hormones were used as competitive substrates. As expected, 5α-DHT and progesterone inhibited, to a large degree α -ZOL production. The production of (β-ZOL. was reduced only in the presence of pregnenolone, but a perceived increase occurred in the presence of two ketosteroids. These results document that there is a decreased production of α -ZOL as a result of an increased concentration of substrate being available for 3β-HSD or a perceived result of accumulated substrate. These results were also found by [19]; depending on the dose of α -ZOL and β -ZOL, the decreased synthesis of progesterone in granular cell cultures from swine occurred due to the inhibited activity of P450scc and 3β-HSD (Fig. 5).

In addition, [35] confirmed that (α-ZOI is mainly produced during hepatic ZEA biotransformation in swine, whereas in cattle β-ZOL is produced. In sheep liver, as a result of ZEA transformation, mainly α -ZOL. is produced. In chickens, as a result of liver biotransformation, mainly β-ZOL is produced in the presence of both enzymatic fractions, microsomal and postmitochondrial, which is consistent with previous studies done on poultry hepatocytes. The result of ZEA biotransformation in the liver of rats is mainly β-ZOL. The studies confirmed previous authors suggestions that different ZEA biotransformation effects are present in different animal species, which can also suggest differences in the distribution of HSDs in ceils. These changes were demonstrated in a comparison of results obtained from enzyme activity studies in microsomal and postmitochondrial fractions. From this work, it is clear that the engagement of 3α - HSD and 3β-HSD in ZEA biotransformation processes is not identical in different animal species. In people, the 3 α - HSD isoforms play a basic role in bile acid biosynthesis in the liver and the hepatic clearance of steroidal hormones. The 3 α -HSDs in humans regulate androgen receptor saturation by transforming active androgen 5α -dehydrotestosterone into 3α - diole, which is a weak androgen and has low affinity for androgen receptors (Steckelbroeck et al. 2004). It is the opposite with 3β-HSD (3β-HSD2 and 3β-HSD3) isoforms, which are the main active steroid hormone synthesis mediators; the hydroxysteroid form is converted into the ketole form, the active receptor.

It is clear that the speed of the glucuronisation process depends on the activity of urydyno-difos- forane glucoronic transferase (UDPGT) in the endoplasmic reticulum, urydyno5'diphosphoro-D-glucoronic acid (UDPGA) availability, and the efficiency of the glucuronisation process toward ZEA, which has been established in only some animal species. For instance, in swine, full glucuronisation has been studied only in liver samples tested with a low. 10 uM. concentration of ZEA, whereas in other animal species the level of glucuronisation was very low. from these studies, it is clear that, in swine, the dominating final form is α-ZOL, which correlates with the higher sensitivity of these animals to ZEA as estrogenous factor [45]. The high concentration of (β-ZOL. not causing estrogenisation effects, which was found in poultry liver samples, confirms the low sensitivity of this animal species (Fig. 4).

Showed the engagement of 3 α - HSD and 3β-HSD during the synthesis and inactivation of many steroids and their expression not only in the liver but also other steroidal tissues, for instance bile ducts or granular cells of the ovaries, in swine or cattle. They also documented that, with no substrate, the expression of HSDs is very low in granular ceils. In their summary, the authors suggest that the results show for the first Lime that ZEA biotransformation occurs outside the liver in the granular cells of swine and cattle. The results correlate with previous conclusions about microsomal and hepatic postmitochondrial HSD fractions in swine and cattle. Microsomes from swine livers mainly produce α -ZOL, whereas the same microsomes in cattle mainly produce β-ZOL. The domination by one metabolite can explain the sensitivity to the presence of ZEA in the above mentioned species, remembering that α - ZOL exhibits higher estrogenic activity than β-ZOL and the initial substance, ZEA.

Consequently, different animal species transform ZEA to α -ZOL to different degrees, which can be estimated as a bioactivation reaction, and transformation to β-ZOL. should be understood as a deactivation reaction. The reduction reaction likely takes place in the intestines (Cavret and Lecoeur 2006), in parallel to the conjugation reaction in the liver, which is preceded by oral ZEA or the administration of its metabolites with feed.

Currently, two paths of ZEA biotransformation are distinguished in animals . The first pathway is ZEA reduction with α - ZOL and β - ZOL production, catalysed by 3 α -HSD and 3β-HSD, respectively. The second pathway is the conjugation of ZEA and its reduced metabolites with glucoronic acid, catalysed by UDPGT. As a result of the different extrapolations of the ZEA biotransformation process, it can be presumed that there is third path of biotransformation, protein antiporter activity in enterocytes with the participation of P glycoprotein or drug resistance protein, which is defined as phase III of detoxication (Fig. 4.). This process consists of excess ZEA being ejected by ion pumps (efflux pumps) outside of the cell. The ZEA molecule returns to the intestinal lumen and is taken up again by enterocytes. This system allows for the organism to be protected from increased ZEA and metabolite concentrations in the cytosol and its consequences [2], but also reduces the amount of toxic substance entering the liver [8].

After analysing the considerations, arguments, and suggestions of different authors concerning ZEA biotransformation processes or its role as a factor disturbing endocrine functions in different animal species, it can be suggested that (i) different animal species show individual sensitivity to ZEA as a result of different biotransformation courses and (ii) the results of different authors and their conclusions give a basis for understanding ZEA biotransformation as far as ZEA transformation to α-ZOL in the liver can . be considered a bioactivation reaction and ZEA transformation to β-ZOL can be considered a detoxication reaction.

Summary

Humans and animals are continuously exposed to different unwanted actions of compounds, including mycotoxins present in food, water, and air. The presence of mycotoxins is a reason for different disease states or tissue and organism dysfunction. New work should facilitate a more accurate description of the degree of species sensitivity and more efficient dietary management, using environmental influences to improve plant material and animal health quality during primary production.

On the other hand, better knowledge of the different forms of enzymes participating in mycotoxin biotransformation would allow more efficient preventive and therapeutic actions in many disease states in which the aetiology .was not fully understood until now.

Conclusions

The results of different investigations confirm the existence of processes of mycotoxin biotransformation in physiologically efficient alimentary lines with:

(i) Diverse microflora that participate in the processes specific for the animal species;

(ii) A different degree of accessibility for the biological mycotoxin;.

(iii) Processes of absorption for the individual mycotoxins in different sections of the alimentary tract for different animal species.'

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