Practice No. 6

Mycotoxins

Mycotoxins:

- Micromycetas are microscopic fungi which are saprophytes
- They are heterotrophic, they don't use photosynthesis
- Most of them produce toxic agents called mycotoxins
- These are products of secondary metabolism and their production depends on many factors as temperature, humidity, substrate etc.
- Very stable in many conditions, esp. termostable
- We know quite well what are the signs of acute exposition to mycotoxins
- But there are also chronic expositions and not only by alimentary route, but also by inhalation. We don't know much about that, but we predict their harmful effect (for example aflatoxins are surely carcinogenic)

- There are more than 300 mycotoxins, but only 20 of them are toxicologically important
- Laboratory diagnosis of mycotoxins is not very easy, there are a lot of them, many metabolites, and methods are rather expensive and difficult (HPLC and gas chromatography)
- Moreover, mycotoxins are not homogenously distributed in the substrate, so proper sampling is important
- Treatment of mycotoxin poisoning is only symptomatic and removing the bad quality feed and prevention is vital
- Legislative limits for animal feed are only assessed for ergot and aflatoxins

- Mycotoxicosis has been known since ancient times
- There were a lot of poisonings from ergot alkaloids
- Ergot (cockle) is product of *Claviceps purpurea*, mould parasite on rye. It looks like a changed enlarged grain and contains mycotoxins called ergot alkaloids
- Acute poisoning causes Convulsive form - convulsions, abortions, hallucinations, maniacal depression, etc. because these mycotoxins stimulate dopamine receptors
- Chronic poisoning is called Gangrenous form due to prevailing vasoconstriction and ischaemia
- Since 18th century, wheat and potatoes became more important foodstuffs in Europe than rye, so these poisoning are rare nowadays

Aflatoxins

- Aflatoxins are the most known and most spread mycotoxins in southern Europe and all warm areas of the world
- Discovered in 1960 in the Great Britain while the Turkey X disease was examined
- Found that it was not an infectious disease, but poisoning by mycotoxins from contaminated feed which was imported from tropical countries
- Produced by *Aspergillus flavus, A. nomius, A. parasiticus*
- 4 aflatoxins - B1, B2, G1, G2

- Toxicity of aflatoxins:
  B1 > G1 > B2 > G2, others less toxic
- The best substrates for their production are cereals and oily crops
- They are very termostable, they bear up temperatures above 250 °C

Metabolism and mechanism of action:

- In an organism, they are metabolised by cytochrome P450 into unstable epoxide derivates. Part of them bind covalently to DNA (forming adducts) and lead to genotoxic and carcinogenic effects. Then changed by hydroxylation to less toxic aflatoxins M1, M2, GM1, GM2, which are excreted not only in urine and faeces, but can be found also in milk, meat and eggs
- All aflatoxins are hepatotoxic and immunosuppresive, and sometimes also damage GIT or kidneys

Ochratoxins

- The most potent ochratoxin is ochratoxin A (OTA)
- Produced by microscopic fungi families of *Penicilium* and *Aspergillus*
- Optimal temperature for growth is around 25°C for *Penicilium* and 30-40 °C for *Aspergillus*, but *Penicilium* grows and produces toxins also in 6 °C in a fridge if there is enough humidity and proper substrate
- Only in ruminants small doses of ochratoxins are destroyed by bacterial microflora in paunch/rumen, all other species are susceptible
- Ochratoxin A causes pig disease called Porcine mycotoxic nephropathy and on Balkan peninsula probably human disease called Balkan endemic nephropathy

Mechanism of action:

- Ochratoxins block proteosyntesis due to exchange with phenylalanine during formation of respective tRNA and during protein chain formation. Also probably increase oxidative stress
- Ochratoxins are primarily nephrotoxic, but can affect also GIT and liver
- During chronic exposition act as immunosuppressive, teratogenic and potentially carcinogenic agents
- They undergo enterohepatic cycle and persist in the body for approximately 1 month

Patulin

- Produced also by *Penicilium* and *Aspergillus*, most common is *Penicilium expansum*
- Substrates - apples and less often other fruits (soft rottening of fruits)
- Spores of *P. expansum* live naturaly on the surface of fruit and don't cause any harm
- But after the damage of the fruit (mechanical or by insects) they get to the flesh of the fruit, germinate, start to grow inside and produce patulin
- The content of patulin is measured especially in food for children (fruit snacks etc.)
- It is destroyed in temperatures above 80 °C (pure patulin, but in fruits it needs approx. 120 °C for 20 minutes, as it is stabilised by vitamin C) and during alcohol fermentation

Mechanism of action:

- Patulin binds to –SH groups of proteins and changes functions of enzymes and proteins, increases permeability of cell membranes
- Acute poisoning is seen in farm animals fed with waste from cider houses etc. and include GIT and neurological signs.
- In humans and some herbivorous pet animals chronic poisoning is more possible and involves teratogenic and immunosuppresive effects

Tremorgenic mycotoxins

- Produced also by *Penicilium* and *Aspergillus*, most common is *Penicilium roqueforti*
- Substrates are cereals, nuts, fruits, but mainly spoiled food like bakery, pasta, cheese, meat products, silage etc.
- Toxins produces are penitrem, paspalinin and most often roquefortin
- Poisoning is typical in dogs and is sometimes called garbage poisoning

Mechanism of action:

- Inhibition of inhibitory neurotransmitters GABA and glycine, or stimulation of receptors for excitatory neurotransmitter glutamate. All mechanisms lead to hyperexcitation
- Clinical signs may involve GIT signs like vomiting and diarrhoea, but are typical with long lasting (hours to days) and hard to treat tremors and convulsions

Fusarium mycotoxins

- *Fusarium* is another genus of moulds and produces a lot of different mycotoxins
- The substrates are usually cereals, legumes and vegetables
- We distinguish three main groups of *Fusarium* mycotoxins: trichothecens, zearalenon, fumonisines
- In contaminated food, there are often many of them together. Their combination is worse than effect of each one separate

Trichothecens:

- Dozens of agents, the most common are deoxynivalenol (DON, former name vomitoxin), T2 toxin and DAS
- Absorbed very well and fast, metabolised and rather quickly excreted by urine and excrements (1-3 days)
- Don’t stay as a residue in tissues so can be assessed only in food/feed

Mechanism of action:

- They all inhibit proteosynthesis, usually by inhibiting the enzyme peptidyltransferase, which is important for the elongation of protein chain. Some also induce apoptosis, damage DNA and RNA and mitochondrial function (celullar breathing and energy production).
- They affect mainly quickly growing and reproducing cells
- The effects are: Gastrotoxicity - vomiting, diarrhoea, inappetence, malnutrition; haematotoxicity – damage of haematopoetic and lymphatic tissues; immunosuppression, dermatotoxicity – damage of hair folicules and skin cells

Zearalenon:

- Also called F2 toxin
- Its typical substrate is maize (corn – *Zea mays*)
- Especially sensitive is swine, other species more or less resistant
- Not fatal poisoning, but long term effects as zearalenone undergoes enterohepatic cycling

Mechanism of action:

- Significant estrogenic effect
- Causes vulvovaginitis, swelling of nipples, disturbs sexual hormones cycle, problems with conception, abortions etc.

Fumonisines:

- Mainly on maize in Mediterranean area and USA
- All species can be affected, but fatal outcome is typical for horses and pigs

Mechanism of action:

- Interference with sphingolipid metabolism. Lacking sphingolipids are problem for membrane function of all cells
- In all species liver damage, where sphingolipids are synthesized, appears
- Horses are sensitive to lack of sphingolipids in myelin sheaths in neurons and suffer from a disease called ELEM (Equine leucoencephalomalacia) which results in paralysis of muscles and failure of vital centres in brain
- Pigs suffer from lack of sphingolipids in myocardial cells and develop cardiac failure with lung oedema (PPE – porcine pulmonary oedema) and die from suffocation
- Fumonisines are also promoters of carcinogenesis and tumours of oesophagus and liver were observed after chronic intake of minimal doses

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Test (from lessons 1-6)