

## 15 General Conclusion

This work focused on aspects related to aflatoxins absorption, biotransformation and excretion in milk of dairy cows. Also the use of mycotoxin adsorbents in dairy diets was studied *in vitro* and *in vivo* conditions to better understand their action mechanisms and improve their sequestering efficiency.

Data presented showed that aflatoxin B1 and other parent molecules (aflatoxin B2, aflatoxin G1 and aflatoxin G2) were quickly adsorbed through gastro-intestinal membranes of ruminants and then transferred to blood and other biological fluids. The speed with which aflatoxins appeared in blood is probably related to an early absorption that could take place in mouth or oesophageal mucous membranes and, successively, in the rumen compartment and in the intestinal tract. The appearance in blood of the principal aflatoxin B1 metabolite, the aflatoxin M1, just after five minutes from the consumption of an oral contaminated drench could represent another evidence of a quick absorption of the aflatoxins. However, this could reasonably establish that the oxidative system responsible of the aflatoxin B1 oxidation, which is present in these tissues and in the leukocytes, is active instantly after absorption.

The passive passage of aflatoxins through membranes was confirmed to be the most probable mechanism involved in absorption of these compounds, as proved by passage of the toxins through non absorbing mucosa like the vagina mucosa.

When absorbed by lactating dairy cows, part of aflatoxin B1 was transferred to the milk as aflatoxin M1. The aflatoxin M1 in milk increased as soon as in the first milking after initial aflatoxin B1 ingestion, up to a plateau observed after 6-7 days of continuous ingestion. The carry over of aflatoxin B1 in milk as aflatoxin M1 is known to be affected by several factors (species, animal variability, aflatoxin source, milk yield, stage of lactation and membrane permeability). The data proposed in this work support the idea that milk yield is the major factor affecting the total excretion of AFM1 and its carry over in milk of dairy cows, while changes of membrane permeability due to inflammatory process do not appear as a factor affecting significantly AFB1 carry over into milk.

The reported carry over values were lower than previously reported data from bibliography, which are in agreement with calculations used to define limits proposed by European Community for aflatoxin B1 in animal feedstuffs and aflatoxin M1 in milk.

Different commercial mycotoxins sequestering agents, usually used in dairy farms, were studied either *in vitro* or *in vivo* conditions. Data from the *in vitro* trial suggested that the circumstances in which these types of experiment were conducted (type of aflatoxins, solution media, dilution factor or aflatoxins:volume, aflatoxins:adsorbents ratio and pH experimental conditions) could strongly influence the sequestering efficacy of adsorbents *in vitro* and cause a different interpretation of results in diverse experimental conditions. Using the rumen fluid as solution media for *in vitro* evaluation, there was an increase in sequestering efficacy due to a probable synergism between rumen fluid, microorganism and adsorbents. This could cause the reduction of aflatoxins absorption when these binders are used on animals.

Testing the strength of the binding between adsorbents and aflatoxins directly in gastro intestinal tract of lactating dairy cows through the formation of an aflatoxin:adsorbent complex, the data clearly showed no correspondence in sequestering efficiency between *in vitro* and *in vivo* trial. This method was proposed to determinate the strength of bind and the fate of aflatoxin:adsorbents complex in gastro intestinal tract in the ruminants.

However, the addition of adsorbents to feedstuffs was shown to reduce the carry over of aflatoxin B1 in milk as aflatoxin M1. More importantly, it appears that the method and time of addition of the adsorbent into the feeds could further increase sequestering efficiency and improve the adsorbents performance. The physical processing methods (mixing or pelletizing) had an effect in improving the amount of aflatoxin B1 being sequestered more so than the time of inclusion of the adsorbents into mixer wagon at the moment of unifeed preparation. In addition, results supported the idea that a higher aflatoxin:adsorbent ratio can improve the effect of sequestering agents in reducing the aflatoxins B1 available for absorption in the gastro-intestinal tract.

Results from the trial have showed that the aflatoxins extraction methodologies usually used in laboratory practices (methanol or acetone water solutions) did not work similarly to determinate aflatoxins concentration in feeds in presence of sequestering agents. This could results in a mistake in analytic determination of aflatoxins level in feedstuff.