Meet the mycotoxin menace head on

WHETHER THE RESULT OF CLIMATE CHANGE OR JUST THE GLOBAL TRADE IN FEEDSTUFFS, MYCOTOXINS HAVE BECOME A SERIOUS THREAT TO PRODUCTIVITY. ONE OF THE MAJOR HURDLES THOUGH, HAS BEEN ESTABLISHING RAPID, RELIABLE AND SENSITIVE TESTING METHODS. BY ANDERS HESTNER.

About the author

Anders Hestner has an M.Sc. in chemical engineering and works as product manager at Diffchamb, who he joined two years ago. Based in Gothenburg, Sweden, Anders' main area of responsibility includes detection methods for mycotoxins, antibiotics and anabolic residues in food and animal feed, working closely with colleagues and distributors world-wide to ensure that customers enjoy a high level of technical assistance.

ELISA testing offers:

- Simple and rapid screening
- Sensitivity in line with EU regulations
- Specific methods for several mycotoxins
- One extraction, several mycotoxins
- Validated methods
- Flexible format and robust design

For more information on mycotoxin testing kits, visit www.diffchamb.com

The threat from mycotoxins to animal and human health is an old problem. Known cases of severe human illnesses caused by mycotoxins go back to the Middle Ages. The economic consequences of mycotoxin contamination and the growing public health awareness have resulted in a great deal of research, risk assessments and development of practices to limit the problem. Regulations on maximum permitted levels in feed and food are in effect in many countries.

Mycotoxins are secondary metabolites of fungi that, in animal feed, are mainly present in groundnuts, beans, peas and grains, such as corn, wheat, barley and oats. Some mycotoxin producing fungi mainly infect crops in the field, e.g. *Fusarium*. Others, such as *Aspergillus* and *Penicillium*, are classified as storage fungi. The optimal growth conditions vary between fungi. Production of aflatoxins by *Aspergillus* is generally favoured by hot and humid conditions, whereas *Penicillium* mycotoxins are produced at moderate temperatures. Consequently, there are geographical differences in contamination levels and composition. The levels also vary between harvests due to variations in climate. Practices to limit and control contamination are adapted to local agricultural conditions. Effective testing methods aid in avoiding dangerous mycotoxin levels in feed and food.

QUALITY CONTROL IS THE KEY

Controlling the storage conditions of raw materials and finished feed by limiting exposure to heat and moisture helps limit mycotoxin problems. Raw material quality control is another crucial step. Efficient quality control calls for simple and rapid screening. The greatest challenges in mycotoxin testing lie in the heterogeneous distribution of the contamination and the low levels of contamination, measured at µg/kg levels. This corresponds to parts per billion (ppb) or 1 second in 32 years! There are many mycotoxin analysis technologies in use, including High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC) and various Enzymatic Immunochemical Assays (EIA).

SENSITIVE TESTING

Enzyme Linked ImmunoSorbent Assays (ELISA), utilise antibodies raised against specific mycotoxins. The methods facilitate detection of small amounts of mycotoxins; aflatoxin B_1 is detected at a level of 0.5 µg/kg. Ground samples are extracted with water or a methanol-water mixture and filtered. Thereafter, the extract is added to a microtitre plate with reaction wells coated with capture antibodies. A mycotoxin conjugate– a mycotoxin bound to an enzyme– and the specific antibody are then added. Competition for specific anti-mycotoxin antibodies starts between the conjugate and the mycotoxin in the sample (*Figure 1*).

In the reaction, mycotoxin and conjugate are captured by the specific antibody, which is fixed to the coated antibody. The excess of conjugate is washed away (*Figure 2*) and a colorimetric substrate is added. The conjugates bind to the antibody colours the substrate blue Adding an acid solution stops the reaction. The blue colour changes to yellow and the absorption of light in the reaction well is measured. The absorption is proportional to the amount of conjugate. This means that the higher the concentration of mycotoxin is, the weaker the yellow colour. The total time for a test is approximately 20 minutes. To quantify the amount of mycotoxin in the sample, a range of standards containing known amounts of mycotoxin is analysed and a standard curve is produced. From this, the concentration of mycotoxin in each sample can be calculated from the absorption in the samples. ELISA detection kits for mycotoxins are supplied in a flexible format allowing a small or large series of samples to be tested. The products can be used several times by storing the reagents refrigerated. <-

FIGURE 1 - SPECIFIC ANTIBODY BINDS TO CAPTURE ANTIBODY. MYCOTOXIN AND CONJUGATE COMPETE FOR THE SPECIFIC ANTIBODY.

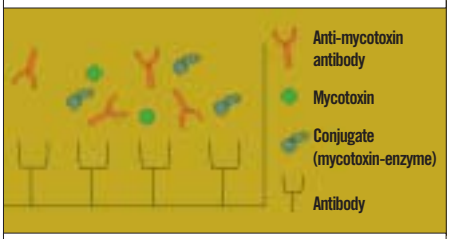

FIGURE 2 - THE MYCOTOXIN AND THE CONJUGATE BOUND TO THE ANTIBODIES AFTER THE WASHING STEP