Understanding mycotoxins in turkeys: Part 2

A rigorous testing schedule should be put in place to continuously assess the mycotoxin threat to the feed stuff and also assist in identifying contaminated lots.

by Marcus Kenny, Company Nutritionist, Aviagen Turkeys. www.aviagenturkeys.com

There is significant variability in the process of testing for mycotoxins brought about by the variability in sampling, sample preparation and analytical variation. Table 1 shows the variability associated in measuring aflatoxin in a lot of contaminated corn. Variation through sampling contributes to over 75% of the overall error of testing.

Sampling error is large because of the extreme distribution among contaminated particles within a lot.

It is estimated that only six kernels in 10,000 are contaminated in a lot containing a concentration of 20ppb aflatoxin.

A single spot sample or probing point is satisfactory if the contaminated particles are evenly distributed through the lot, however mycotoxins generally occur in isolated pockets through the lot. Increasing the number of samples taken from a lot can increase the chances of identifying contaminated lots. Procedures used to take a sample from a bulk lot are extremely important; every individual item in the lot should have an equal chance of being chosen.

The sample should be an accumulation of many small portions taken from many different locations throughout the lot. When drawing a sample from a bulk container a probing pattern should be developed so that product can be collected from different locations in the lot. An example of a probing pattern used by the USDA is shown in Fig. 1.

The sampling probe should be long enough to reach the bottom of the container when possible. When sampling from a moving stream, for example a moving belt, small increments should be taken along the entire length of the moving stream.

Composite all the increments to obtain a bulk sample. If the bulk sample is larger than required then blend and subdivide the bulk sample to obtain the desired size test sample.

Fig. 1. An example of a 5 and 8-probe sampling pattern (adapted from Whitaker et al., 2005), X = 5 Probe Patterns; X+O = 8 Probe Patterns.

Front		
X	O	
		X
	X	O
O		X
X		
Rear		

Analyses

Rapid strip tests:
Analyses of feedstuffs for presence of mycotoxins can be conducted efficiently through the use of enzyme linked immunosorbent assays (ELISAs) testing kits and have become a standard tool for rapid monitoring of mycotoxins. This method is satisfactory in order to establish if a specific feedstuff is either under or over a legal compliance level.

HPLC and GC-MS analyses provides more accurate determination of the level and type of mycotoxins present in the feedstuff.

Fig. 2. Artemia salina.

Some toxins can escape detection as they may be masked by glycosides or proteins which are attached to the toxin giving a false negative result, more refined analyses methods are required to measure such toxins.

Bio-assays are used to establish the presence of specific mycotoxins. An example is using crustacea, such as Artemia salina (see Fig. 2), and assessing survival rate from a sample of material.

Preventative approaches

Assessing the mould levels of grain can indicate the likelihood of mycotoxins occurring.

Testing the material for the level and type of mould can sometimes indicate what the likelihood of mycotoxin contamination may be. However it is possible that moulds may no longer be present in the material but the mycotoxins are; the best practice is to analyse for both moulds and mycotoxins.

Damage or stress to the plant by diseases, insect or bird damage, weeds, frost or drought permits easy entrance of moulds and fungi, and promotes rapid development of moulds.

Insect damaged grain is more vulnerable to mould growth so reducing insect infestations is critical in preventing mould growth in grains. Some toxins such as aflatoxins tend to occur in broken and damaged kernels and in foreign material.

Avoid harvesting grain at an excessively high moisture content and keep in a holding bin using forced air to keep cool, store the grain in weatherproof, well ventilated facilities and monitor the temperature of stored grain.

Drying the grain slowly and at low temperatures for long periods promotes aflatoxin development.

All handling equipment and storage facilities must be kept well ventilated and clean and dry prior to and during use.

Storage facilities must be free of moisture leaks and all residue removed to reduce contamination.

Applying liquid or dry mould inhibitors, use of organic acids such as propionic acid and ammonium isobutyrate will prevent mould growth if correctly applied as it is augured into the silo.

However, organic acids will not destroy toxins already present in the grain.

Continued on page 13

Table 1. The variability measured by the variance associated with a 0.91kg sample, 50g subsample, measuring aflatoxin in 1 aliquot by immunoassay in a lot of shelled corn at 20ppb aflatoxin[1].

	Variance	Ratio (%)
Sample = 0.91kg	268	75.5
Subsample[2], 50g	56	15.9
Immunoassay, 1 aliquot	30	8.6
Total	355	100

[1]Sampling, sample preparation, and analyses errors account for about 75.5, 15.89 and 8.6% of the total errors, respectively. [2]Romer Mill used to grind.

T-2 toxin (ppm)	Egg production (%)	Egg weight (g)	Body weight (g)
0.0	96.29	52.45	1,332
0.5	93.81	51.77	1,313
1.0	91.75	51.35	1,286
2.0	86.65	51.33	1,285

Table 2. The effect of T-2 toxin on laying hen performance.

Continued from page 11

Visual assessment of the lot

Look out for visual clues of contaminants. Grains can show signs of mould growth (see Fig. 3) and/or insect damage and presence of 'fines' which are associated with mould growth.

Cleaning

During the cleaning process of contaminated grain, dust, husks, hair and shallow particles are blown away by aspiration or scouring. Grain cleaners have been shown to reduce the level of aflatoxin in maize grain by as much as 50%.

Mechanical sorting and separation

In this process the clean product is separated from mycotoxin-contaminated grains. High feed losses are possible due to incomplete and uncertain separation. Therefore mechanical sorting and separation is not always considered cost-efficient. 'Blending down' material which has been analysed higher than maximum permitted levels of toxins is not permitted in some regions.

Washing

Washing procedures using water or sodium carbonate solution result in some reduction of mycotoxins in grains.

Treatment:

Nutritional approaches:

- Increased levels of antioxidants, methionine, selenium and vitamins in affected feeds have been shown to counteract the effects of mycotoxins as well as addition of chlorophyll, algal derivatives and aspartamine.

Chemical detoxification:

- Detoxification with ammonia or ammonia related compounds is considered to be one of the most practical means of decontamination of aflatoxin in agricultural commodities. Dietary aflatoxin inactivation by ammonisation for layer breeders had no detrimental effect on the immunological response elicited by Newcastle disease vaccination as measured by haemagglutination-inhibition titers. Hydrogen peroxide is an oxidising agent acceptable in foods and has the potential to destroy up to 97% of aflatoxins.

  Similar effects have been found with treatment by organic acids and surfactants.

Mycotoxin sequestering agents:
Supplementation with non-nutritive mycotoxin-sequestering agents is by far the most practical and most widely studied method for reducing the effects of mycotoxin exposure.

- Activated charcoal is an amorphous form of carbon heated in the absence of air and then treated with oxygen to increase porosity. There is some data to suggest activated charcoal is effective in absorbing some aflatoxins but not toxins derived from other species.

  Activated charcoal can also result in absorption of micronutrients in the feed.

- Silicate minerals (clays) include bentonite, zeolite and hydrated sodium calcium aluminosilicate.

  The absorption technique uses compounds that form a complex with the toxin preventing absorption of the aflatoxin across the intestinal epithelium reducing the amount of toxin absorbed into the bloodstream.

  High levels of inclusion could provide excessive sequestration capacity that may decrease the bioavailability of important micronutrients.

Fig. 3. Maize grains contaminated with mould.

- Yeast cell wall-based adsorbents, principally modified glucomannan, are able to adsorb higher levels of several mycotoxins at lower inclusion rates than inorganic binders.

  The specific mode of action of some yeast cell wall components suggests that their activity would not affect the availability of micro-nutrients. Modified glucomannan has been shown to bind fusarium derived toxin.

Biotransformation

Biological detoxification by enzymes and/or micro-organisms degrades mycotoxins within the gastro-intestinal tract, before resorption into the animal occurs.

There are now enzyme and micro-organism based products effective in transforming specific toxins such as fumonisins and trichothecenes into non-toxic metabolites.

Summary

- Prevent fungal growth on crops in the field, at harvest, during storage of feedstuffs and processing of feed.
- Implement mechanical means of removing contaminated material from the feedstuff and consider addition of mould inhibitors/killers.
- Implement a mycotoxin testing schedule. This is important for the risk assessment to livestock and also from a regulatory and human health point of view.
- Apply a robust sampling plan. Increasing the number and size of samples taken from a lot can increase the effectiveness of testing and the chances of identifying contamination.
- Detect and quantify the mould and mycotoxin concentration in the feed-stuff remembering many mycotoxins co-contaminate materials. Detection of one toxin may indicate presence of another more toxic mycotoxin.
- When the feedstuff is contaminated take action before the birds consume the feed, not after the birds are affected by the toxin.
- Remove and replace the feed or apply an appropriate mycotoxin binder or bio-transforming agent specific to the type of toxin recovered in the feed.
- Monitor the flock for any performance or clinical related signs of mycotoxicosis.
- Implement an ongoing mycotoxin surveillance programme. ■

References are available from the author on request

Table 3. Co-contaminating mycotoxins in poultry (adapted from Devegowda and Murthy, 2005).

	Aflatoxin	DAS	DON	Fumonisin B	Fusaric acid	Ochratoxin	T-2 toxin
Aflatoxin		++	+	-	-	++	++
DAS	++		-	+	-	-	++
DON	+	-		-	++	-	-
Fumonisin B	-	+	-		-	-	+
Fusaric acid	-	-	++	-		-	-
Ochratoxin	++	-	-	-	-		++
T-2 toxin	++	++	-	+	-	++	

+ signifies an additive effect of toxins, ++ signifies a synergistic effect. - no known additive or synergistic effect