AMINONews®

By Trevor K. Smith, Ph.D., P.Ag.,
Adjunct Professor, Dept. of Animal Biosciences,
University of Guelph,Guelph, Ontario, Canada, N1G 2W1; tsmith@uoguelph.ca

Dietary Mycotoxins: Impact on Gut Health and Performance of Poultry

Introduction

Mycotoxins are harmful metabolites produced by molds (fungi). They can originate pre-harvest due to adverse climatic conditions and can continue to be formed postharvest under suboptimal storage conditions. The most significant mold species producing mycotoxins pre-harvest include *Aspergillus* and *Fusarium* fungi while *Penicillium* and also *Aspergillus* fungi are more significant with respect to contamination in storage. While environmental conditions in storage of feedstuffs can be controlled, climatic conditions pre-harvest can present a significant challenge to producers.

It would appear that the frequency of poultry productivity challenges arising from mycotoxin-contaminated feeds is increasing. This is likely due, in part, to extreme weather conditions in crop growing areas globally. The United Nations Intergovernmental Panel On Climate Change recently reported that global warming and extreme weather conditions are becoming more frequent. High moisture conditions arising from excess rainfall and flooding pre-harvest are key factors in promoting mold growth and potential mycotoxin contamination. Drought conditions can also increase the chances of mycotoxin contamination, however, as shriveling and cracking of grain kernels can allow fungal spores to breach fibrous mechanical barriers and to grow on starchy endosperm layers. The spectrum of mycotoxins produced under hot, dry, drought conditions (eg aflatoxin, fumonisin), however, usually differs from those produced under wet, cool conditions (eg. deoxynivalenol (DON), zearalenone, fusaric acid).

Recent Trends in Mycotoxin Research

Advances in Analytical Methodology

Our understanding of the significance of the effects of feed-borne mycotoxins in poultry production has largely paralleled advances in analytical chemistry as applied to feed analysis. A major advance in identifying mycotoxin-contaminated feedstuffs is the increasing use of LC/MS/MS methodology. This technique utilizes liquid chromatography coupled to two mass spectrometers linked in tandem. LC/MS/MS is a very sensitive methodology but a unique characteristic is the ability to quantify multiple mycotoxins and metabolites simultaneously. This has increased our awareness of potential additive and synergistic effects of different mycotoxins which was previously a largely theoretical concept. This helps us understand why responses of poultry to contaminated feedstuffs are often more severe than would be expected based on concentrations of individual mycotoxins. The ability to analyze several hundred compounds in one run is now possible. This will likely prove to be the greatest advance in our understanding of mycotoxin challenges to poultry production since the advent of ELISA quick test kits. The ELISA kits are rapid and inexpensive which permitted much more extensive monitoring of raw materials and finished feeds compared to techniques such as TLC or HPLC. The limitation of the ELISA kits is their specificity. Kits analyze for only one mycotoxin per kit using antibody technology. This limits the spectrum of compounds that can be analyzed to the availability of commercial kits. Commercial LC/MS/MS services are increasingly available

and offer varying degrees of specificity depending on price. The high capital and labor costs of LC/MS/MS technology has, heretofore, limited its application in poultry feedstuffs. Costs are continually declining, however, due to methodological advances.

Emerging Mycotoxins

Survey data arising from the use of LC/MS/MS has pointed out the frequency of multiple mycotoxin co-contamination of naturally-contaminated feedstuffs. It is now possible to quantify contamination by mycotoxins that were seldom analyzed for previously. This was often due to the lack of commercially available ELISA test kits. These previously underreported mycotoxins are now referred to as “emerging mycotoxins” (Gruber-Dorninger *et al.*, 2016). Some examples are: enniatins, beauvericin, moniliformin, fusaproliferin, fusaric acid, culmorin, butenolide, sterigmatocystin, emodin, mycophenolic acid, alternariol and tenuazonic acid. Little is known about the toxicity of many of these compounds to poultry. An example of applying the power of LC/MS/MS technology is the report of Blandino *et al.* (2017) in which corn samples grown in Italy over several crop years under natural conditions were analyzed and 37 different mycotoxins and metabolites were detected. All samples contained fumonisin, fusaric acid, fusaproliferin, DON, DON-3-glucose, culmorin and zearalenone. Concentrations of aurofusarin correlated with DON while concentrations of moniliformin and beauvericin correlated with fumonisin. Such multiple co-contamination points to the possibility of significant additive and synergistic effects of mycotoxins which could exaggerate the response of poultry compared to that expected with the challenge of individual mycotoxins.
This concept was further supported by the studies of Andretta *et al.* (2016) who conducted a meta-analysis of 85 published studies of the feeding of mycotoxin-contaminated diets to growing pigs. The feeding of diets contaminated with individual mycotoxins reduced feed intake and weight gain by 14 % and 17 % respectively. The feeding of diets contaminated with multiple mycotoxins, however, reduced feed intake and weight gain by 42 % and 45 % respectively. This again stresses the potential significance of additive and synergistic effects when diets are contaminated with multiple mycotoxins.

Conjugated (Masked) Mycotoxins

This important topic has recently been reviewed by Berthiller *et al.* (2013). It appears that some plants have the ability to chemically modify mycotoxins produced by invading fungi. This may be a natural defense mechanism as some mycotoxins are also phytotoxins. The natural occurrence of conjugated mycotoxins has been reviewed by Zhang *et al.* (2019). Some of these conjugated mycotoxins that were first reported include DON-3-glucose (Berthiller *et al.*, 2005) and zearalenone-4-glucose (Schneweis *et al.*, 2002). More recently, glucose conjugates of fumonisin, T-2 toxin, nivalenol and fusarenon-X have also been reported. This is a rapidly moving field of research and sulfate conjugates of DON have recently been reported in wheat (Warth *et al.*, 2015). Such compounds are not detectable using conventional analytical techniques such as ELISA and HPLC and are, therefore, sometimes referred to as being “masked”. The relative toxicity of such compounds to poultry is still not clear, however, as there is potential for enzymes in bacteria in the digestive tract to hydrolyze the conjugate to generate free mycotoxins which may then enter the blood stream and attack target tissues. Much research needs to be conducted to determine the range of conjugated mycotoxins that can be found in naturally-contaminated feedstuffs. Once this is determined, the relative toxicity of the conjugated and free forms of mycotoxins must be determined in different types of poultry. It will also be necessary to have access to analytical standards of the conjugates to determine their frequency in naturally-contaminated feeds. It is likely that conjugated mycotoxins contribute to unexpected toxicity in poultry based on conventional feed analysis.

The Significance of Multiple Mycotoxin Contamination of Feedstuffs

With the advent of LC/MS/MS technology, it is clear that multiple mycotoxin contamination is the most common characteristic of naturally-contaminated feedstuffs. Even though many mycotoxins may be detected in very low concentrations, many mycotoxins have similar mechanisms of action in poultry. An example is immunosuppression. Aflatoxin, fumonisin, DON, T-2 toxin, fusaric acid and many other common mycotoxins are to varying degrees immunosuppressive in poultry. Immunosuppression can result in lingering health problems in the flock, birds that do not respond to medications and even failure of vaccination programs. The net effect can be increased mortalities. The lesions that are seen post-mortem are not, however, classical mycotoxin lesions. They are lesions caused by the infectious organism that took advantage of the impaired defense mechanisms of the bird. Mortality is only indirectly caused by mycotoxins. We then rely on chemical analysis of feed to determine if mycotoxins are a factor and often the concentrations detected should not cause mortalities based on literature reports of studies with individual purified mycotoxins. This is complicated by the possible presence of non-detectable conjugated (masked) mycotoxins and also the additive effects of emerging mycotoxins not normally analyzed for.

In addition to possible additive effects, multiple co-contaminants can also lead to synergistic effects. This is the circumstance where the presence of one mycotoxin can increase the toxicity of another mycotoxin. An example is the interaction between DON and fusaric acid (Smith *et al.*, 1997). Onc characteristic of the toxicity of DON and other trichothecenes such as T-2 toxin is increased blood amino acid concentrations. This is thought to be due to the ability of DON to inhibit liver protein synthesis. Free amino acids are then released into the blood at elevated concentrations including tryptophan. Blood tryptophan can then cross the blood-brain barrier and lead to increased synthesis of the neurotransmitter serotonin, which has a sedative-like effect. Fusaric acid has no effect on liver protein synthesis, but when contaminated diets are consumed, fusaric acid enters the blood. Fusaric acid has a chemical structure similar to tryptophan. Tryptophan is a bit unusual compared to other amino acids in that it is transported in blood not in free form but mainly loosely attached to albumin protein. Fusaric acid can, therefore, compete with tryptophan for binding sites on albumin and can displace tryptophan to increase the free tryptophan concentration in blood. Only free tryptophan can cross the blood-brain barrier to serve as a substrate for serotonin synthesis. The pathway for serotonin synthesis is poorly regulated and increasing concentrations of tryptophan in the brain results in increased serotonin synthesis and increases the behavioral changes such as reduced feed intake, loss of muscle coordination and lethargy (Smith and MacDonald, 1991). DON, therefore, increases blood tryptophan concentrations leading to increased brain serotonin synthesis and altered behavior but fusaric acid multiplies this effect by increasing brain uptake of free tryptophan. This is an example of toxicological synergy arising from multiple mycotoxin co-contamination of feeds.

Effects of Feed-Borne Mycotoxins on Poultry

Major Mycotoxins and Their Modes of Action

(a) Aspergillus and Penicillium Mycotoxins

Aflatoxin

Alfatoxin was one of the first mycotoxins associated with a significant disease outbreak in poultry. Widespread mortality was suddenly observed in the U.K. turkey flock in 1960. The condition was described as Turkey X Disease. The cause of the outbreak was eventually identified as a shipment of alfatoxin-contaminated Brazilian peanut meal. Aflatoxin is produced mainly by the fungi *Aspergillus flavus*, hence the name aflatoxin, and *Aspertillus parasiticus.* These are considered tropical or semi-tropical fungi which thrive under warm, moist conditions. The major chemical forms of aflatoxin are B_1, B_2, G_1 and G_2 of which aflatoxin B_1 is the most common . The terms B and G refer to the blue and green fluorescence emitted by these compounds when exposed to UV

light. The liver is the primary target organ in acute aflatoxicosis which is thought to be caused mainly by the parent compound. Extensive fatty infiltration and liver enlargement is seen. This is likely due to inhibition of liver protein synthesis and reduced mobilization of liver lipids due to inhibition of the synthesis of lipid transporting proteins. Immunosuppression is also seen.
An example of aflatoxicosis in poultry is provided by the work of Azizpour and Moghadam (2015). Broiler chicks were fed diets with and without 250 ppb aflatoxin in naturally-contaminated diets for 35 days. The feeding of the contaminated diet caused liver enlargement as well as significant liver changes including fatty infiltration. Blood triglyceride, uric acid and cholesterol concentrations were reduced and activities of the enzymes aspartate aminotransferase and alanine aminotransferase were increased. These are symptoms of liver damage.
Aflatoxin has likely received more attention from the scientific community than other mycotoxins, however, due to the carcinogenic nature of aflatoxin metabolites. The liver is again the main target organ and there is considerable concern about aflatoxin residues in foods of plant and animal origin from a human public health standpoint.

Ochratoxin A

Ochratoxin A is produced mainly by *Aspergillus ochraceus* and *Penicillium viridicatum*. Ochratoxin A is considered to be a mycotoxin produced mainly during grain storage which allows for a greater degree of control compared to mycotoxins produced pre-harvest. The target organ for this compound is the kidney resulting in elevated blood uric acid concentrations and extreme gout. Immunosuppression is also seen. Although less so than aflatoxin, ochratoxin A is also carcinogenic and can cause kidney tumors.

(b) *Fusarium Mycotoxins*

Fusarium fungi thrive in more temperate climates and globally are likely the most significant family of feed-borne mycotoxins from the standpoint of poultry production. The relative proportions of different *Fusarium* mycotoxins varies, however, depending on temperature and moisture conditions during the growing season pre-harvest.

Fumonisin

The fumonisins are a relatively small family of more recently discovered *Fusarium* mycotoxins. The most common and toxic form is fumonisin B_1 (FB_1). Fumonisin is most commonly found in corn and corn by-products and is produced by *Fusarium verticillioides* (*moniliforme*) and *Fusarium proliferatum* and tends to contaminate crops produced under warmer and dryer conditions compared to some other *Fusarium* mycotoxins. The chemical structure of fumonisin allows it to inhibit the synthesis of lipids causing reduced function of biological membranes. Horses are particularly sensitive to feed-borne fumonisin with as little as 3 ppm causing mortalities due to brain lesions. Poultry are much more resistant and the feeding of 200 ppm fumonisin derived from fungal culture material was required to reduce growth rates of broilers and turkeys. Such high levels of contamination are not found in commercial poultry rations but this does not mean, however, that fumonisin is insignificant in poultry production because fumonisin is also immunosuppressive. This can contribute to the additive effect of multiple co-contaminants on flock health.

Trichothecenes

The trichothecenes are a group of over 100 structurally-related mycotoxins the most common of which are deoxynivlaenol (DON, vomitoxin), T-2 toxin, HT-2 toxin, nivalenol, fusarenon – X and diacetoxyscirpenol (DAS). Trichothecenes are produced by *Fusarium tricinctum*, *Fusarium verticillioides* (*moniliforme*) and *Fusarium solani*. Early studies focused mainly on T-2 toxin which has a high relative toxicity and is relatively easy to produce in purified form from fungal culture material. Acute doses of T-2 toxin have been shown to inhibit liver protein

synthesis leading to high blood amino acid concentrations (Meloche and Smith, 1995). The mucosa of the digestive tract is a target organ and oral lesions have been seen in poultry fed diets containing 0.4 ppm T-2 toxin as well as gizzard erosion and necrosis of proventricular mucosa.

The effect on intestinal morphology of growing turkeys fed diets naturally-contaminated with DON, zearalenone and fusaric acid was determined by Girish and Smith (2008a, Table 1). Day-old turkey poults were fed diets for 12 weeks with intestinal measurements made after 3, 6, 9 and 12 weeks including villus height, crypt depth, villus width, thickness of submucosa and muscularis, villus-to-crypt ratio and apparent villus surface area, which correlates with nutrient absorption. After 3 weeks of feeding, contaminated diets decreased villus height in the duodenum and jejunum and apparent villus surface area in the jejunum. Duodenal villus width and apparent villus surface area and jejunal villus height and apparent villus surface area were reduced after 6 weeks of feeding the contaminated diets. No effects of diet were seen after 9 and 12 weeks of feeding. It was concluded that diets contaminated with multiple *Fusarium* mycotoxin co-contaminants could adversely affect intestinal integrity and nutrient uptake in poultry. The effect of feeding DON-contaminated diets on duodenal structure in turkeys was confirmed by Devreese *et al.* (2013). These authors also used LC/MS/MS technology to directly measure concentrations of DON and DOM-1, a DON metabolite, as a direct measure of exposure of birds to DON-contaminated diets. More recently, Wang and Hogan (2019) reported reduced illeal villus heights in broilers fed diets containing up to 7.9 ppm DON from contaminated wheat with growth reduced in the grower phase as previously reported by Swamy *et al.* (2004a).

Table 1: Effects of feeding *Fusarium* mycotoxins on small intestinal morphology of turkeys[1]

Starter Phase (0-3 wks)			
Duodenum			
Diet	**Villus Height (µm)**	**Villus Width (µm)**	**Villus Surface Area ($µm^2$)**
Control	968.4[a,2]	64.7	62,103
Contaminated	782.7[b]	64.5	51,953
Jejunum			
Control	448.0[a]	66.5	29,944[a]
Contaminated	380.0[b]	62.0	23,604[b]

[1] Girish and Smith (2008a)
[2] Means with different superscripts are significantly different ($P \leq 0.05$).

In addition to harmful effects on digestive tract integrity, trichothecene mycotoxins have also been associated with behavioral changes in poultry, especially reduced appetite. Pigs are particularly sensitive to trichothecene-induced feed refusal which may also be associated with vomiting, hence vomitoxin, a common term for DON. Poultry are generally less sensitive than pigs. This species difference has been attributed to different effects of contaminated feed on brain neurochemistry. Swamy *et al.* (2004b) observed that consumption of contaminated feeds elevated brain concentrations of the neurotransmitter serotonin in both species but broiler chickens also were observed to also have an elevation in brain concentrations of the neurotransmitter norepinephrine, which may reduce the effect of serotonin on feed intake. Swamy *et al.* (2002a, 2004a) fed blends of grains naturally-contaminated with *Fusarium* mycotoxins, largely DON, and observed that reduced feed intake and growth depression occurred in the grower phase (4 – 6 weeks) but not in the starter phase (1 – 3 weeks). This observation was recently confirmed by Wang and Hogan (2019). A comparative study of the effects of trichothecene-contaminated diets on brain neurochemistary of laying hens, broiler breeders and turkeys indicated that laying hens were the most sensitive group with respect to elevations in brain concentrations of the neurotransmitter serotonin (Yegani *et al.*, 2006a). Subsequent studies of Girish *et al.* (2008b) showed that the

feeding of such diets to turkeys adversely altered the nervous system in some regions of the brain.
A third mode of action of trichothecenes is immunosuppression. This can again contribute to the additive effects of numerous mycotoxins on health status of poultry. Girish *et al.* (2008c) reported immunosuppression in turkeys fed a blend of grains naturally-contaminated with multiple mycotoxins, mainly DON. Sensitivity to dinitrochlorobenzene, which triggers an immune response on contact with skin, was decreased after 24 and 72hours by feedborne mycotoxins when compared to controls.

Zearalenone

Zearalenone is primarily produced by *Fusarium graminearum* and *Fusarium roseum*. Although not chemically an estrogen, zearalenone can bind to estrogen binding sites in the reproductive tract resulting in an estrogenic syndrome. Zearalenone has little effect on behaviors such as appetite suppression and is not immunosuppressive. Zearalenone promotes growth of some tissues especially the uterus. This can result in rectal and vaginal prolapse, infertility and increased frequency of abortions in swine, the most sensitive species. Poultry are much less sensitive to zearalenone toxicosis. Performance and reproduction are not affected at typical levels of contamination. Increased frequency of prolapse can, however, be seen in laying hens.

Fusaric Acid

Fusaric acid is a common *Fusarium* mycotoxin that has received relatively little attention from poultry scientists in the past. This is perhaps because of the low acute toxicity of this compound. It does, however, have effects on the nervous system and this is the key to its significance physiologically. Considerable research into the pharmacological effects of fusaric acid was conducted by the Japanese pharmaceutical industry. Using a variety of experimental models, fusaric acid was shown to inhibit brain enzymes resulting in reduced concentrations of the neurotransmitter norepinephrine (Nagatsu *et al.*, 1970). The resulting effect was a loss of blood pressure and this was of interest to the pharmaceutical industry as a potential treatment for high blood pressure in humans (Hidaka *et al.*, 1969). Such a loss of blood pressure can reduce blood flow between organ systems and cause tissue edema in poultry.
Perhaps of greater interest, however, was the observation that fusaric acid could cause increased brain concentrations of the neurotransmitter serotonin (Smith and MacDonald, 1991). This was then the basis for the previously described toxicological synergy between fusaric acid and DON.
A third mode of action of fusaric acid is that like trichothecenes, fumonisin, aflatoxin and many other mycotoxins, fusaric acid is also immunosuppressive. This again points to the significance of additive effects of multiple mycotoxin contamination on poultry flock health.

Effects of Mycotoxins on Avian Intestinal Immunity

The feeding of grains naturally-contaminated with multiple *Fusarium* mycotoxins, largely DON, to broiler chickens resulted in a significant reduction in bile immunoglobulin A concentrations after 56 days of feeding (Swamy *et al.*, 2002a). Bile is quite rich in IgA which protects the digestive tract from pathogens such as e. coli. Bile IgA concentrations were also significantly reduced in laying hens fed similarly contaminated diets (Chowdhury *et al.*, 2005).
The effects of feeding turkey poults diets naturally-contaminated with *Fusarium* mycotoxins, largely DON, on intestinal immunity and gut health were reported by Girish *et al.* (2010). It was concluded that the feeding of the contaminated diet adversely affected gut immunity and mucosal cell development.

Antibiotic Effects of Mycotoxins and Potential Effects on Intestinal Microbiota

The effects of mycotoxins on gut health and intestinal microbiota have recently been reviewed by Liew and Mohd-Redzwan (2018). Feed-borne mycotoxins can alter gut microbiota composition reducing beneficial bacteria and increasing gut pathogens.
A potentially significant effect of feed-borne mycotoxins on intestinal microbiota arises from their antibiotic properties. *Penicillium roqueforti* fungi can synthesize many mycotoxins including patulin, mycophenolic acid, roquefortine C, PR toxin and penicillic acid. Penicillic acid, mycophenolic acid and PR toxin have been shown to have antimicrobial effects (Kopp-Holtwiesche and Rehm, 1990). Patulin has been explored as an antibiotic in human and veterinary medicine (Madhyastha *et al.*, 1994). Such *Penicillium* fungi, however, are considered to be storage molds and do not invade field crops pre-harvest. These antibiotic mycotoxins are most commonly found in the silage component of ruminant diets and appear to be very rare in poultry feeds. Increased use of LC/MS/MS technology in mycotoxin analysis of monogastric feedstuffs has, however, led to more frequent detection of small amounts of these compounds.

Dietary Influences on Mycotoxicoses in Poultry

Dietary Protein and Amino Acids

Dietary protein levels can have a significant effect on mycotoxic diseases in poultry and other species. Mycotoxic diseases are often characterized by changes in metabolic utilization of dietary protein and increased dietary protein is often required for normal growth and development (Smith, 1982). Aflatoxicosis is an interesting example. Acute aflatoxicosis is characterized in poultry by reduced growth rate and fatty infiltration of the liver and these symptoms are due to the direct toxic effect of the parent compound. Dietary protein supplementation can reduce the effects of acute aflatoxicosis by increasing the activity of enzymes that promote aflatoxin breakdown. Aflatoxin breakdown products, however, are carcinogenic and high protein diets, therefore, promote the liver tumors characteristic of chronic aflatoxicosis.
Dietary supplementation with individual amino acids can also alter symptoms of mycotoxic diseases. Acute oral doses of T-2 toxin were shown to increase brain concentrations of tryptophan and the neurotransmitter serotonin in weanling rats (MacDonald *et al.*, 1988). Supplementing a tryptophan-deficient, gelatin-based diet with a mixture of large neutral amino acids (leucine, isoleucine, valine, tyrosine and phenylalanine) reduced brain concentrations of tryptophan and serotonin in control-fed rats but this was not enough to overcome the acute effects seen in T-2 toxin-treated animals (Cavan *et al.*, 1988). Administration of T-2 toxin did not alter the free- to- protein bound ratio of blood tryptophan in contrast to reports for fusaric acid (Chaouloff *et al.*, 1986) but total blood tryptophan concentrations were elevated. It was concluded that though acute doses of both T-2 toxin and fusaric acid result in increased brain concentrations of the neurotransmitter serotonin, the mechanisms by which this occurs differs.
A meta-analysis of the interaction between diet and mycotoxins in papers published on broiler performance was conducted by Andretta *et al.* (2011). Ninety-eight papers were used totalling 37,371 birds. Ochratoxins and aflatoxins had the greatest negative effects on feed intake and growth rates reducing feed intake by 17 % and 11 % respectively and reducing weight gain by 20 % and 11 % respectively. Reductions in broiler weight gain caused by aflatoxins was 0.23 % lower with each 1g increased in daily protein ingestion and 7.3 % lower with each 1-g increased in daily methionine ingestion. This likely reflects the role of methionine in aflatoxin detoxification as described above.

Dietary Prebiotics, Probiotics and Enzymes

These dietary supplements act to prevent mycotoxicoses through the action of microorganisms capable of inactivating mycotoxins enzymatically. Such supplements are generally referred to as mycotoxin inactivators. This topic has recently been reviewed by Murugesan *et al.* (2015). Awad *et al.* (2009) fed broilers the prebiotic inulin,

which increased glucose absorption in the jejunum and colon of birds treated with DON. This was attributed to the selective promotion of bacteria that produce short-chain fatty acids which increase serum glucose transporters. The inclusion of probiotic *Eubacterium* to a broiler diet containing 10 ppm DON prevented DON-induced intestinal damage (Awad *et al.*, 2006). Diaz (2002) reported that supplementing broiler diets of birds fed 1 ppm of 4,15-diacetoxyscirpenol with encapsulated bacterium prevented adverse effects.
The feeding of polyvinylpolypyrrolidone, which has epoxidase and lactonase enzyme activity, prevented the decrease of peripheral immune cells in broiler chickens fed 2.5 ppm aflatoxin B_1 (Celik *et al.*, 2000). Supplementing diets with these enzymes, however, did not prevent the adverse effects of DON and zearalenone on performance, metabolism and immunity of broilers (Danicke *et al.*, 2003). A challenge to the use of such enzymes to detoxify mycotoxins is their specificity. The wide range of chemical structures of free and conjugated mycotoxins would require multiple enzyme supplements to be effective when poultry are challenged with multiple co-contaminants.

Mycotoxin Adsorbents

Mycotoxin adsorbents are high molecular weight, non-nutritive, non-fermentable polymers that act like non-digestible fibres that pass down the digestive tract of poultry and adsorb small molecules to prevent absorption into the blood stream and transfer to target tissues. The advantage of the adsorbent approach is that such polymers are very non-specific and are therefore well suited to diets containing multiple mycotoxins. A challenge, however, is to arrive at the appropriate level of dietary inclusion to prevent mycotoxic diseases. Under supplementation will not completely prevent harmful effects. Over supplementation of poultry diets with adsorbents must be avoided, however, as the non-specific nature of these materials means that they can also adsorb vitamins, minerals, amino acids and intestinal digestive secretions such as bile salts, thereby causing nutritional deficiencies .
Mycotoxin adsorbents can be classified as being either inorganic or organic polymers. The inorganic adsorbents are silica-based polymers and examples are zeolites, bentonites and diatomaceous earth. Organic adsorbents are carbon-based and can include lignin-rich fibers, activated charcoal and yeast cell wall extracts. Silica-based adsorbents have been shown to be mainly effective against aflatoxin but they can still be effective in overcoming *Fusarium* mycotoxicoses at higher levels of dietary inclusion. The feeding of bentonite to rats exposed to diets containing T-2 toxin resulted in decreased intestinal absorption and increased fecal excretion of T-2 toxin (Carson and Smith, 1983a). Similar results were seen with the feeding of fibre-rich alfalfa meal (Carson and Smith, 1983b).
The feeding of a glucomannan based, yeast cell wall-based adsorbent was effective at preventing reduced egg production, elevated blood uric acid concentrations and reduced bile immunoglobulin A concentrations in laying hens fed grains naturally-contaminated with multiple *Fusarium* co-contaminants (Chowdhury and Smith, 2004). In a similar study with broiler breeders, this adsorbent prevented reduced egg shell thickness in fertilized eggs and reduced early embryonic mortality in birds fed diets naturally-contaminated with multiple *Fusarium* mycotoxins (Yegani *et al.*, 2006b, Table 2). In a study of the effects of diets naturally-contaminated with multiple *Fusarium* mycotoxins fed to broiler breeder pullets challenged with coccidiosis, Girgis *et al.* (2010) reported that the feeding of contaminated diets altered immune status of the digestive tract. Similar effects were seen when the glucomannan-rich adsorbent was fed in the absence of mycotoxins indicating that such adsorbents have immunostimmulatory potential in the digestive tract regardless of the degree of dietary mycotoxin contamination.

Table2: Effects of feeding *Fusarium* mycotoxins on reproductive performance of broiler breeder hens[1]

Diet	Shell Thickness (µm)	Early Embryonic Mortality (%)	Hatchability (%)
Control	32.1[a]	5.4[a]	76.5[a]
Contaminated	30.1[b]	21.5[b]	68.7[a]
Contaminated + Adsorbent	31.5[a]	2.3[a]	89.2[a]

[1] Yegani et. al. (2006b)
[2]Means with different superscripts significantly different (P<0.05).

Conclusions

It appears that the frequency of mycotoxin challenges to the global poultry industry is increasing. This is due to unfavorable extreme weather conditions in crop growing areas which can lead to increased mold growth and mycotoxin production pre-harvest. The ability to detect feed-borne mycotoxins has been greatly advanced by the use of LC/MS/MS technology. This technique is very sensitive and allows simultaneous quantification of far more compounds and metabolites than was previously possible. Recent publications using this technique have confirmed the presence of “emerging” mycotoxins which may contribute to additive and synergistic effects resulting from multiple mycotoxin co-contamination of feedstuffs. Adding to the complexity of feed analysis is the presence of more recently discovered conjugated or “masked” mycotoxins which may be toxic to poultry but not detected by conventional analytical techniques. Mycotoxins can impair gut health by damaging the integrity of the digestive tract through structural changes, by impairing mucosal immunity and by acting as antibiotics. Mycotoxins can reduce poultry production efficiency by altering behavior such as reducing feed intake due to changes in brain chemistry, causing immunosuppression resulting in poor flock health status, reducing nutrient utilization and impairing reproduction. Many adverse effects of mycotoxins in poultry can be at least partially overcome by supplements of dietary protein and amino acids, prebiotics, probiotics, enzymes and mycotoxin adsorbents. This is a very active and ongoing field of research with new information and solutions being continually generated to ensure poultry production efficiency despite the challenges posed by mycotoxin contaminated diets.

Literature Cited

Andretta, I., Kipper, M., Lehnen, C.R., Hauschild, L., Vale, M.M. and Lovatto, P.A. 2011. Meta-analytical study of productive and nutritional interactions of mycotoxins in broilers. Poult. Sci. 90: 1934 – 1940.

Andretta, I., Kipper, J., Hauschild, L., Lehnen, C. R., Remus, A. and Melchior, R. 2016. Meta-analysis of individual and combined effects of mycotoxins on growing pigs. Sci. Agric. 73: 328 – 331.

Awad, W.A., Bohm, J., Razzazi-Fazeli, E., Ghareeb, K. and Zentek, J. 2006. Effect of addition of a probiotic micro-organism to broiler biets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult. Sci. 85: 974 – 979.

Awad, W.A., Ghareeb, K. and Bohm. J. 2009. Animal feed additive and the effect of the *Fusarium* toxin deoxynivalenol on the electrophysiological measurement of transepithelial ion transport of young chickens with Ussing chamber technique. Int. J. Poult. Sci. 8: 25 – 27.

Azizpour, A. and Moghadam, N. 2015. Effects of yeast glucomannana and sodium bentonite on the toxicity of aflatoxin in broilers. Braz. J. Poult. Sci. 17: 7 – 13.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G. and Krska, R. 2005. Masked mycotoxins: Determination of a deoxynivalenol glucoside in articially and naturally contaminated wheat by liquid chromatography – tandem mass spectrometry. J. Agric. Food Chem. 53: 3421 – 3425.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S.D., Haesaert, G., Karlovsky, P. Oswald, I.P., Seefelder, W., Speijers, G. and Stroka, J. 2013. Masked mycotoxins: a review. Mol. Nutr. Food Res. 57: 165 – 186.

Blandino, M., Scarpino, V., Giordano, D., Sulyok, M., Krska, R., Vanara, F. and Reyneri, A. 2017. Impact of sowing time, hybrid and environmental conditions on the contamination of maize by emerging mycotoxins and fungal metabolites. Ital. J. Agron. 12: 928 pp 215 – 224.

Carson, M.S. and Smith, T.K. 1983a. Role of bentonite in prevention of T-2 toxicosis in rats. J. Anim. Sci. 57: 1498 – 1506.

Carson, M. S. and Smith, T.K. 1983b. Effict of feeding alfalfa and refined plant fibers on the toxicity and metabolism of T-2 toxin in rats. J. Nutr. 113: 304 – 313.

Cavan, K.R., MacDonald, E.J. and Smith, T.K. 1988. Potential for dietary amino acid precursors of neurotransmitters to overcome neurochemical changes in acute T-2 toxicosis in rats. J. Nutr. 118: 901 – 907.

Celik, I., Oguz, H., Demet, O., Donmez, H.H., Boydak, M. and Sur. E. 2000. Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. Br. Poult. Sci., 41: 430 – 439.

Chaouloff, F., Laude, D., Merino, D., Serrurrier, B. and Elghozi, J.L. 1986. Peripheral and central short-term effect of fusaric acid, a dopamine-beta-hydroxylase inhibitor, on tryptophan and serotonin metabolism in the rat. J. Neural Transm. 63: 219 – 232.

Chowdhury, S.R. and Smith, T.K. 2004. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of laying hens. Poult. Sci. 83: 1849 – 1856.

Chowdhury, S.R., Smith, T.K., Boermans, H.J. and Woodward, B. 2005. Effects of feed-borne *Fusarium* mycotoxins on hematology and immunology of laying hens. Poult. Sci. 84: 1841 – 1850.

Danicke, S., Matthes, S., Halle, I., Ueberschar, K.H., Doll, S. and Valenta, H. 2003. Effects of graded levels of Fusarum toxin-contaminated wheat and of a detoxifying agent in broiler diets on performance, nutrient digestibility and blood chemical parameters. Br. Poult. Sci. 44: 113 – 126.

Devreese, M., Girgis, G.N., Tran, S.-T., De Baere, S., De Backer, P., Croubels, S. and Smith, T.K. 2013. The effects of feed-borne *Fusarium* mycotoxins and glucomannan in turkey poults based on specific and non-specific parameters. Food Chem. Toxicol. 63: 69 – 75.

Diaz, G.J. 2002. Evaluation of the efficacy of a feed additive to ameliorate the toxic effects of 4,15-diacetoxiscirpenol in growing chicks. Poult. Sci. 81: 1492 – 1495.

Girish, C.K. and Smith, T.K. 2008a. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on small intestinal morphology of turkeys. Poult. Sci. 87: 1075 – 1082.

Girish, C.K., MacDonald, E.J., Sheinin, M. and Smith, T.K. 2008b. Effects of feedborne *Fusarium* mycotoxins on brain regional neurochemistry of turkeys. Poult. Sci. 87: 1295 – 1302.

Girish, C.K., Smith, T.K., Boermans, H.J. and Karrow, N.A. 2008c. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, hematology, metabolism and immunocompetence of turkeys. Poult. Sci. 87: 421 – 432.

Girish, C.K.,, Smith, T.K., Boermans, H.J., Anil Kumar, P. and Girgis, G.N. 2010. Effects of dietary *Fusarium* mycotoxins on intestinal lymphocyte subset populations, cell proliferation and histological changes in avian lymphoid organs. Food Chem. Toxicol. 48: 3000 – 3007.

Girgis, G.N., Barta, J.R., Girish, C.K., Karrow, N.A., Boermans, H.J. and Smith, T.K. 2010. Vet. Immunol. Immunopath. 138: 218 – 223.

Gruber-Dorninger, C., Novak, B., Nagl, V. and Berthiller F. 2017. Emerging mycotoxins: Beyond traditionally determined food contaminants. J. Agric. Food Chem. 65: 7052 – 7070.

Hidaka, H., Nagatsu, T., Takeya, K., Tatshuisa, T., Suda, H., Kojiri, K., Matsuzaki, M. and Umezawa, H. 1969. J. Antibiot. 22: 228 – 230.

Kopp-Holtwiesche, B. and Rehm, H.J. 1990. Antimicrobial action of roquefortine. J. Environ. Pathol. Toxicol. Oncol. 10: 41 – 44.

Liew, W.P. and Mohd-Radzwan, S. 2018. Mycotoxin: Its impact on gut health and microbiota. Front. Cell. Infect. Microbiol. 8: 60.

MacDonald, E.J., Cavan K.R. and Smith, T.K. 1988. Effect of acute oral doses of T-2 toxin on tissue concentrations of biogenic amines in the rat. J. Anim. Sci. 66: 434 – 441.

Madhyastha, S., Marquardt, R.R., Mast, A., Borsa, J. and Frohlich, A.A. 1994. Comparison of toxicity of different mycotoxins to several species of bacteria and yeast: Use of Bacillus brevis in a disc diffusion assay. J. Food Prot. 57: 48 – 53.

Meloche, J.L. and Smith, T.K. 1995. Altered tissue amino acid metabolism in acute T-2 toxicosis. Proc. Soc. Exper. Biol. Med. 210: 260 – 265.

Murugesan, G.R., Ledoux, D.R., Naehrer, K., Berthiller, F., Applegate, T.J., Grenier, B., Phillips, T.D. and Schatzmayr, G. 2015. Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult. Sci. 94: 1298 – 1315.

Nagatsu, T., Hidaka, H., Kuzuya, H., Takeya, K., Umezawa, H., Takeuchi, T. and Suda, H. 1970. Inhibition of dopamine beta-hydroxylase by fusaric acid (5-butylpicolinic acid) in vitro and in vivo. Biochem. Pharmacol. 19: 35 – 44.

Schneweis, I., Meyer, K., Engelhardt, G. and Bauer, J. 2002. Occurrence of zearalenone-4-beta-D-glucopyranoside in wheat. J. Agric. Food Chem. 50: 1736 – 1738.

Smith, T.K. 1982. Influence of mycotoxins on protein and amino acid utilization. Federation Proc. 41: 2828 – 2832.

Smith, T.K. and MacDonald, E.J. 1991. Effect of fusaric acid on brain regional neurochemistry and vomiting behavior in swine. J. Anim. Sci. 69: 2044 – 2049.

Smith, T.K., McMillan, E.G. and Castillo, J.B. 1997. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75: 2184 – 2191.

Swamy, H.V.L.N., Smith, T.K., Cotter, P.F., Boermans, J.J. and Sefton, A.E. 2002a. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on production and metabolism in broilers. Poult. Sci. 81: 966 - 975.

Swamy, H.V.L.N, Smith, T.K., MacDonald, E.J., Boermans, H.J. and Squires, E.J. 2002b. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 80: 3257 – 3267.

Swamy, H.V.L.N., Smith, T.K., Karrow, N.A. and Boermans, H.J. 2004a. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological parameters of broiler chickens. Poult. Sci. 83: 533 – 543.

Swamy, H.V.L.N., Smith, T.K. and MacDonald, E.J. 2004b. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on brain regional neurochemistry of starter pigs and broiler chickens. J. Anim. Sci. 82: 2131 – 2139.

Wang, A. and Hogan, N.S. 2019. Performance effects of feed-borne *Fusarium* mycotoxins on broiler chickens: Influences of timing and duration of exposure. Anim. Nutr. 5: 32 – 40.

Warth, B., Fruhmann, P., Wiesenberger, G., Kluger, B., Sarkanj, B., Lemmens, M., Hametner, C., Frohlich, J., Adam, G., Krska, R. and Schuhmacher, R. 2015. Deoxynivalenol-sulfates: identification and quantification of novel conjugated (masked) mycotoxins in wheat. Anal. Bioanal. Chem. 407: 1033 – 1039.

Yegani, M., Chowdhury, S.R., Oinas, N., MacDonald, E.J. and Smith, T.K. 2006a. Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on brain regional neurochemistry of laying hens, turkey poults and broiler breeder hens. Poult. Sci. 85: 2117 – 2123.

Yegani, M., Smith, T.K., Leeson, S. and Boermans, H.J. 2006b. Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders. Poult. Sci. 85: 1541 – 1549.

Zhang, Z., Nie, D., Fan, K. Yang, J., Guo, W., Meng, J., Zhao, Z. and Han, Z. 2019. A systematic review of plant-conjugated masked mycotoxins: Occurrence, Toxicology, and metabolism. Crit. Rev. Food Sci. Nutr., DOI: 10.1080/10408398.2019.1578944.

The content of this document is for the information and assistance of Evonik's customers and is not applicable to products marketed or sold in the United States. Evonik markets its products in compliance with applicable local laws, but it is the user's responsibility to ensure that the use of and any claims made for this product comply with all relevant requirements. This document may not be published in the United States without the express written consent of Evonik Operations GmbH.

Evonik Operations GmbH
Animal Nutrition Business Line

animal-nutrition@evonik.com
www.evonik.com/animal-nutrition