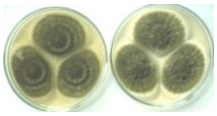


Reducing the Impact of Mycotoxins

Overview

> General Background on Molds & Mycotoxins
> Mycotoxins of Concern for Game Birds
> Managing Mycotoxin Risks

MYCOTOXINS – A few definitions

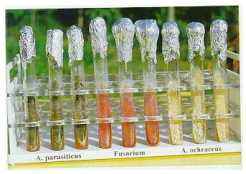
- Myco = fungi (molds are fungi)
- Toxin = toxic biological compound (poison)
- Mycotoxicosis = mycotoxin induced disease
- Mycoses = disease caused by fungi (mold)

TOXICOLOGY: The study of toxins

- First Principle of Toxicology
  - All substances can be poisons
  - Often, dose differentiates a poison and a remedy
- Toxicity:
  - Acute - exposure to chemical for < 24 h
    - Some toxins act very quickly (DON & T-2)
  - Chronic - repeated exposure
    - Some toxins have a longer term effect (Aflatoxin, Ochratoxin)

Mycotoxins are Primarily Produced by These Three Families of Molds

- *Aspergillus species*
  - **Aflatoxins**
  - **Ochratoxins**
- *Fusarium species*
  - **Tricothecenes**
    - **DON, T2, etc.**
  - **Zearalenone**
  - **Fumonisins**
- *Penicillium species*
  - **Ochratoxin**

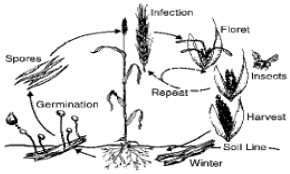


>(Devegowda *et al.*, 1998)

PRINCIPLES OF MOLD GROWTH

Mold Growth Cycle

Molds form spores that reside in the soil and contaminate the plant during the growth cycle of the plant



(NDSU Extension Service)

Requirements for Mold Growth

- Moisture – varies by mold types
- Temperature – varies by mold types
- Oxygen - molds don't grow without it
- Nutrients
  - Carbon sources – sugars, starches, proteins, or fats
  - Nitrogen sources – proteins
  - More easily accessed after processing or damage to grain

Seed Coat is Natural Protection Against Molds

Seed Coat Damage

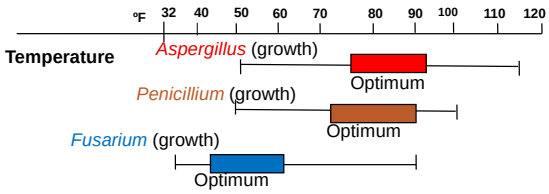
- **Increases fines and broken kernels**
- **Opens up access of molds to nutrients in the kernel**
- **Increases chance of mold infection and mycotoxin production**

(Watson and Ramstad, 1987)

Lower Limits of Moisture Needed for Fungal Growth in Cereal Grains

Species or Group	Minimum Moisture, % w.b.	
	Soybeans	Corn
Aspergillus spp.	12.0 - 12.5	14.0 - 17.0
Penicillum spp.	17.0 - 20.0	16.5 - 20.0
Fusarium		22.0

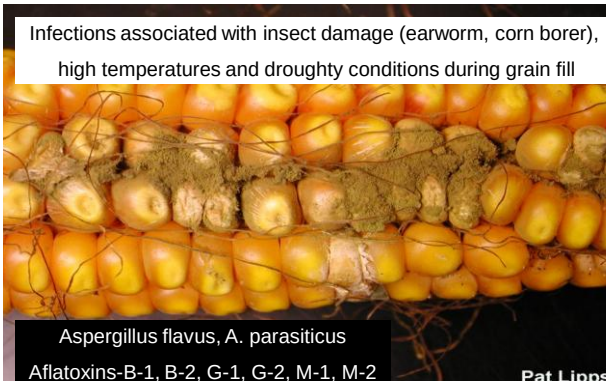
Temperature Ranges for Mold Growth



Field Molds

- ***COOL & WET***
  - More than 20% Moisture
  - Cool after pollination
- *Fusarium spp.*
  - **Fumonisins**
  - **Tricothecenes (DON, DAS, T-2, HT-2,)**
  - **Zearalenone**
- *Penicillium spp.*
  - **Ochratoxins**

- ***HOT & DRY***
  - More than 16% Moisture
  - Hot, dry conditions
  - Often triggered by insect damage
- *Aspergillus species*
  - **Aflatoxins**
  - Also a common storage mold

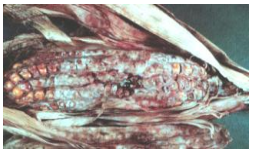


Fusarium species

***Gibberella zeae* ear rot**

>**Reddish-pink to white mold on ears**

>***Fusarium* molds may produce DON (vomitoxin) , zearalenone, T-2, HT-2, DAS**



Cool, wet conditions in 1st 21 days after pollination

Fusarium species

Toxin: Fumonisin

> Warm,dry years

> Insect related kernel damage promotes the disease

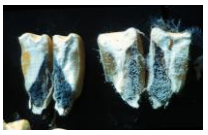
> Less fumonisin in Bt corns

Storability Factors

- > Moisture
  - > Not *average* moisture but moisture of *wettest* kernels in storage
  - > Commonly affected by asymmetric heating in storage structure
- > Temperature
- > Physical damage to the kernel
- > Infection by field molds
- > Oxygen present
  - > Molds don't grow in anaerobic conditions

Storage Molds

- > Grow at moistures of 13-20%
- > *Aspergillus* and *Penicillium*
  - > These molds are ubiquitous; nearly all corn kernels have these spores on their surface
  - > Infection is determined by moisture, temperature, damage to kernel
- > Conditions are rarely favorable for *Fusarium* molds to grow in storage, except in "high moisture" stored grain



Mold Effects on Animals

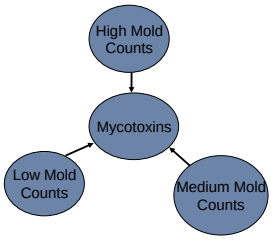
- > Reduce nutrients, especially energy
- > Reduce palatability
- > May be allergenic
- > *Produce toxins as a defense mechanism, when stressed*

Molds Mycotoxins

- Molds & mycotoxins are almost always present in feeds at some, generally very low, level
- Very common for more than one mycotoxin to be present in feedstuffs
- Mycotoxins may be present in feed with low mold levels
- Molds may die, but mycotoxins are stable and remain

Mycotoxin Levels

- The mechanism that causes molds to produce mycotoxin is not fully understood.
- It is generally understood that stress conditions on the mold may trigger mycotoxin production.
- Mold counts are not an indication of mycotoxin production because:
  - A stress condition is required to start mycotoxin production.
  - The death of a toxin-producing mold may give low counts but the toxin may still be present.
  - The same fungus can produce several mycotoxins.
  - Several fungus are able to produce the same mycotoxin.



Major Mycotoxins

Major classes of mycotoxins
- Aflatoxins
- Trichothecenes (DON, T-2, DAS)
- Fumonisins
- Zearalenone
- Ochratoxin

- More than 10,000 different mycotoxins are known, less than 50 are well characterized
- Well defined analytical methods for ~20 mycotoxins
- Commercial quick tests ~ 6

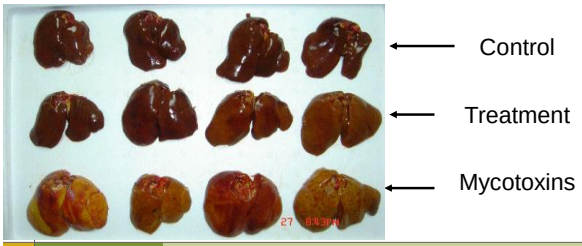
General Effects of Mycotoxins in Birds

- Reduced weight gain and feed efficiency
- Loose droppings
- Reduced egg production, hatching rate, and hatchling viability
- Greater incidence of disease
  - Immune suppression
  - Hidden damage to vital organs

Aflatoxins

- Aflatoxins are produced by *Aspergillus spp.*
- Very toxic to all poultry
- Absorbed from the gut, converted in the liver to more toxic compounds
- Effects
  - Poor gains & feed conversion
  - Reduced egg production
  - Stunting – slow growth, weak immune system
- Ducks, pheasants & partridges are very susceptible
- Quail, are susceptible, but less than other species

Aflatoxin Effects: Chicken livers



Ochratoxins

- Produced by *Aspergillus* & *Penicillium spp.*
- Very toxic to most poultry
  - Attacks the kidneys
  - Reduces intake & gain
  - Poor, slow feathering
  - Reduced egg production, with yellow stain
  - Weak chicks
- Quail and partridges have been shown to be very sensitive
- More common to southern US and other warmer climates

Ochratoxicosis 1200 ppb

Kidney weight increase

Unchallenged	Ochratoxin
Control	Challenged

Fumonisin

> Family of toxins produced by *Fusarium spp.*

> Relatively low toxicity, but at high levels can cause immunosuppression and reduce performance

> Can increase the effects of other toxins, when present in combination

> Last year, southern corn belt had fairly high levels of fumonisin with aflatoxin in corn crop

> Effects in game birds have not been extensively studied

Zearalenone

>Produced by *Fusarium spp.*

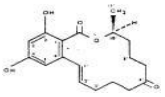
>Not primarily involved in health or performance problems

>Mimics the effect of the female hormone estrogen

> Poultry are generally resistant to Z effects

> At high doses may increase the size or early maturity of reproductive organs and reduce egg production

>Not well understood in game birds



Tricothecenes

- Produced by *Fusarium* spp.
- > 40 toxins identified with similar chemical structure
  - T-2 & HT-2
  - DAS (diacetoxyscipenol)
  - DON (deoxynivalenol, vomitoxin)
- Tricothecenes generally act as tissue irritants and reduce feed intake
- Immunosuppressants at higher doses
- Produce reproductive problems, primarily in poultry
- **T-2 and sometimes DAS are primary concerns for game birds**

T-2 Producing Molds

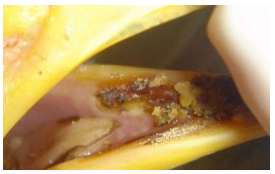
> Fusarium graminearum
> Fusarium roseum
> Fusarium tricnictum
> Fusatriumoxysporum
> Fusarium solani
> Fusarium nivale
> Fusarium lateritium
> Fusarium episphaeri
> **Color**
  > Commonly Pink or Red tint

T-2 – Health Symptoms

- General
  - Reduced Intake & Gain
  - Poor feed efficiency
- Reproduction
  - Delayed puberty
  - Reduced fertility
  - Poorer hatchability
- Decreased Immune Function
  - Increase disease susceptibility
- Gastrointestinal tract
  - Irritation and Hemorrhaging – bloody diarrhea
  - Oral Lesions
  - Necrosis of digestive tract

T-2 toxin effects in broilers

Oral lesions



T-2 Toxin Summary

- Fairly common toxin
- Causes irritation, hemorrhage, and necrosis especially in the mouth
- May reduced bird growth, health and reproduction
- May cause immunosuppression
  - Can lead to secondary effects, especially when combined with other mycotoxins
- T-2 has been shown to affect pheasants, quail, chukars, geese & ducks
- Young birds are especially sensitive

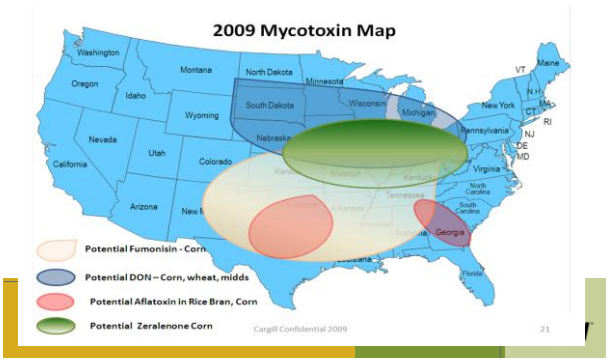
INTERACTIONS

- It is common to find more than one toxin present
- Effects of many toxin combinations are at least additive and may be synergistic
- Combinations of aflatoxin and other toxins are particularly damaging

Summary for Game Birds

- Game birds appear to be more sensitive, in general, to mycotoxins, than domestic poultry species
- Aflatoxin
  - Strong toxin, fairly common, often seen when drought stress occurs
- Ochratoxin
  - Strong toxin, less common
- T-2 Toxin
  - Moderately common, produces lesions in mouth and gut, reduces intake, reduces immunity, interferes with reproduction, produces weak chicks
- Other toxins may cause problems in combinations of toxins, or in combination with other stressors (disease, crowding, weather changes, molting, etc.)

FAO considers 25% of the world's cereal yield is reported contaminated with mycotoxins with an annual variation in the incidence.



General Toxin Trends 2009 Crop

- Level of pre-harvest mold, especially in the midwest was extremely high
  - These moldy grains reduce palatability and nutrient level
- Conditions appeared prime for producing Tricothecenes
- Early reports of DON (vomitoxin); somewhat less in subsequent weeks
  - Maybe a phenomenon of producers trying to "unload" the worst product first?
- Less information related to T-2 available, but vigilance is important for gamebird industry, since T-2 production is commonly produced by the same molds as DON
- Pockets of other toxins

Mycotoxins in DDG's

- DDG's and corn gluten feed typically contain ~3 times more mycotoxin than the source grain
- Mycotoxins are stable to processing
- Removal of starch to make ethanol leaves mycotoxins concentrated in remaining fraction
- Good news- most ethanol producers have increased screening to avoid accepting incoming grain with toxins
- Due to sensitivity of game birds, use of DDG's and corn gluten byproducts should still be limited

Managing Mycotoxins

Feed Suppliers Should Monitor for Mycotoxins

>Understand risks – local information, surveys, known risks

>Sample properly

>Challenging because molds grow in irregular pockets in grain

>Multiple large samples, mix well, sub-sample

>Measure toxins using reliable methods

>Ideally test for toxins *prior* to accepting or unloading grains

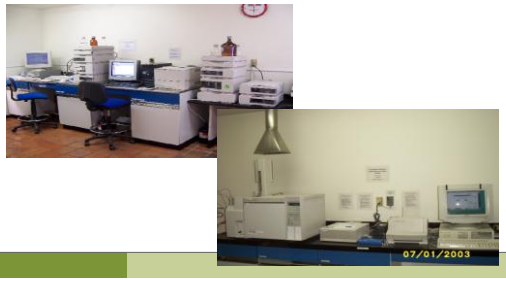
>Quick tests are improving the speed, cost, and quantitative results for mold results

>Monitor according to specific regional challenges

HPLC or GC/MS Analysis

>Reference Standard Methods for Mycotoxins for more than 20 mycotoxins

>Cost per sample is high



>Charm ROSA System

>3-10 minutes

>Simple solvents

>Sensitive & Quantitative

>Tests for 6 key toxins

>Aflatoxin, DON, T-2/HT-2,

>Fumonisin, Zearalenone, OTA

Mycotoxin Management

Feed Supplier Responsibility - Dealing with potential for contamination

- > Develop a proactive program to reduce risk
- > Understand risks unique to species being fed
- > Follow trends in region of sourced feed ingredients
- > Reject loads by analyzing at receiving
  - > Customers Beware – someone else will buy those loads
- > Prevent risky product from entering sensitive feeds
  - > Eliminate or strongly limit DDG's & gluten products in game bird feeds
- > Manage levels in all diets to well below risk levels

Managing Mycotoxins

Grain producer or handler responsibility

>Prevention and Handling of Molds & Mycotoxins

- > Screen moldy grains, remove fines and light weight grains suspected of contamination
- > Dry down to 13 percent moisture
- > Store moldy grain separately
- > Monitor nutrient levels of suspect grain
- >Monitor mycotoxins at feed plant before receiving

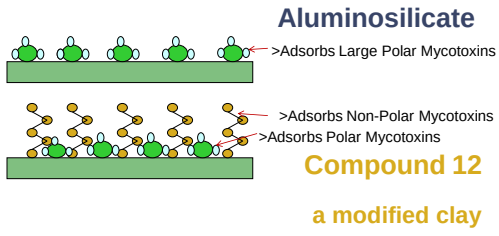
Nutritional Approaches

- > **Under FDA regulations feed ingredients cannot be sold as mycotoxin binders**
- > However some AAFCO listed products have been shown to reduce toxin effects
  - > Tricothecenes (DON, T-2) are commonly the most difficult to manage
- > Super-supplementation of nutrients important to fight stress
  - > Vitamins, trace minerals, amino acids
  - > Effect over normal diets may be limited
- > Immune protecting ingredients
  - > Anti-oxidant nutrients (vitamin E, organic Se)
  - > Appropriate glucan sources

Adsorbing Mycotoxins

- > Some zeolite clays adsorb aflatoxin, but have minimal effects against other toxins
  - > A few are effective against fumonisin
- > Other Approved Ingredient types that help control other mycotoxins
  - > Specially modified clays
  - > Specially processed yeast cell walls (MOS-glucan)
  - > Specially processed charcoal type carbon products
- > Commercial products are evaluated using a combination of animal (*in vivo)* tests and laboratory *(in vitro)* assays
- > Challenging area to research in live animals
  - > Toxins affect all species, but not all species respond the same
  - > Limited animal data; very hard to control levels
  - > Very expensive to conduct – normally animals must be destroyed

Comparison of Compound 12 with Standard Clay as Adsorbents



In vitro Methods

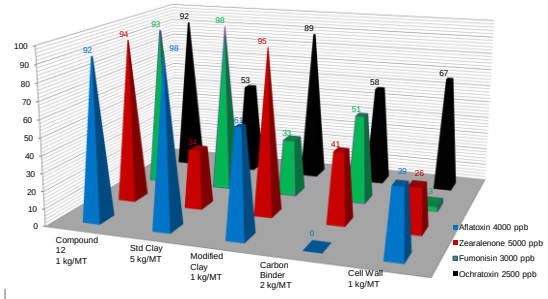
- Provide an estimation of the adsorption capability of the mycotoxin adsorbing ingredient and the probability of animal functionality
- These tests depend on experimentation conditions and should be validated by "*in vivo*" tests
- Useful for comparing products and product dosing

In vitro Measures

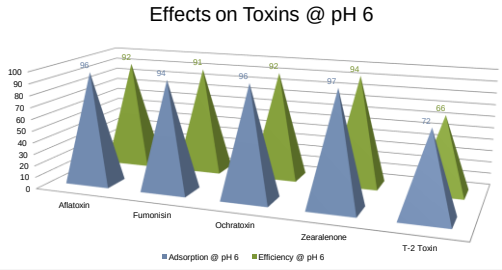
> % Adsorption = % toxin bound
> % Desorption = % bound toxin released after "washing"
> % Efficiency = % Adsorption - % Desorption
> General principle – if it won't bind *in vitro* and stay bound, it won't work in the animal either
> Common problem – reports based on *in vitro* doses of toxins and adsorbing ingredients that don't reflect commercial dosing

In vitro adsorbent evaluation

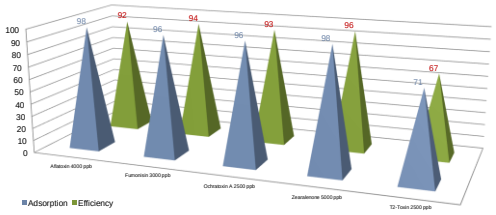
Efficiency of Approved Ingredients vs. 4 Mycotoxins



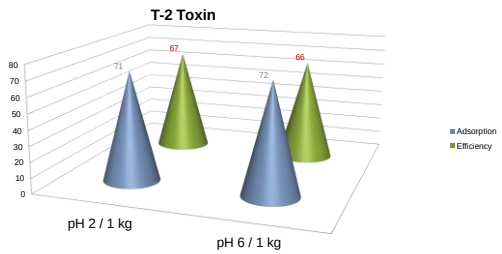
Compound 12



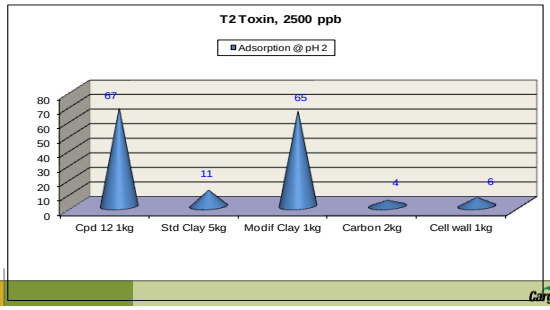
Compound 12 Effects on Toxin@ pH 2



Compound 12 Effects on T-2 Toxins at Two pH Levels



In vitro T2 Toxin Neutralizing Comparison



Products Tested for 2500 ppb T-2 Neutralizing

- High quality standard clay @ 10 lbs/US ton → 11% neutralizing @ pH 2
- MOS-Glucan product @ 2 lbs/US ton → 6% neutralizing @ pH 2
- Modified Carbon @ 4 lbs/US ton → 4% neutralizing @ pH 2
- Alternative Modified Clay @ 2 lbs/US ton → 65% neutralizing @ pH 2
- Combination clay & glucan product @ 2 & 4 lbs/US ton → < 10% neutralizing @ pH 2 and 6
- 2 Experimental feed ingredients (Clay type & Cell Wall type) → < 10% neutralizing @ pH 2 and 6
- **Compound 12 @ 2 lbs/US ton → 67 – 71% neutralizing @ pH 2 and 6**

Compound 12 provides the best neutralizing of toxins

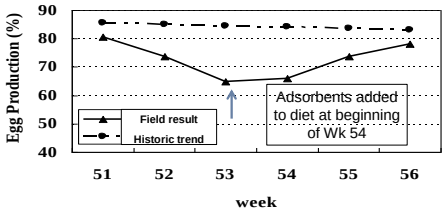
In vivo studies remain the ultimate proof

- Studies may be field studies comparing flocks in fed different treatments
  - Challenge is lack of control or statistical validity
  - Challenge may be more or less than expected
- Controlled studies can be conducted
  - Challenge delivering controlled, but realistic challenge consistently
  - Costs are very high

Field Study – Laying Hens

- A problem of oral lesions in laying hens was presented in a farm located in Southeast Mexico.
- The lesions were observed in a range of gray to black tongue coloring and oral ulcerations.
- The egg production decreased from 80.6% to 64% for 53 week old hens.
- No apparent disease was observed and the oral lesions were related with the feed quality.
- Measurement showed some low toxin levels

Egg production recorded weekly



It was observed that after the egg production reached the lowest level at the 53rd week Compound 12 was added to diets, it began to increase, reaching almost the standard level at the 56th week. The recovery in egg production began within 1 week of starting the treatment with the adsorbents.

Controlled Challenge Study

Effect of trichothecenes in broilers and evaluation of the efficiency of Compound 12 adsorbent

Objective of the experiment

> To evaluate the efficiency of Compound 12 adsorbent, to avoid the development of lesions of birds when consuming trichothecenes contaminated feeds.

Material and Methods

> 96 one day old male Hubbard broilers
> 4 week study
> Performance and histology measures
> Trichothecenes grown from one *Fusarium sporotrichioides* strain.
> The concentration of trichothecenes in the diets was confirmed by quantification via GC/MS.
> 4 treatments (T), of 8 birds with 3 repetitions.

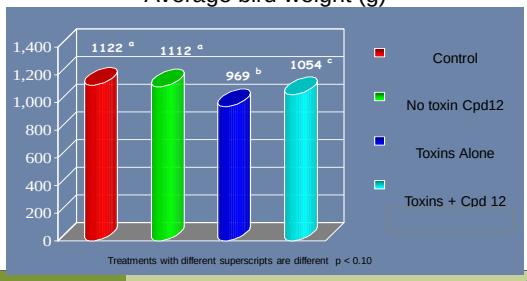
Materials & Methods

Experimental diets:

(1) control diet, without adsorbent nor trichothecenes
(2) innocuity diet: control + 1.5 kg/t of Compound 12
(3) diet with mycotoxins: T-2 toxin -1170 ppb, H T-2-330 ppb, tetraol- 4000 ppb, neosolaniol- 1400 ppb
(4) challenge diet (toxin + adsorbent): T-2 toxin-1100, H T-2 -310 ppb, tetraol-3600 ppb, neosolaniol-1200 ppb) + 1.5 kg/t of Compound 12

Results

Average bird weight (g)



Results

> The results showed statistically significant differences between some of the groups in weight and feed conversion, which are asociated with the consumption of these mycotoxins.

Results

14 days of trichothecenes consumption


Mycotoxins **Mycotoxins + Cpd 12**


Conclusions

> Severe characteristic lesions were observed with the presence of these mycotoxins in oral cavity, oropharynx & proventriculus for the diet with mycotoxins alone

> The inclusion of Compound 12 into the contaminated diet improved animal weight and feed conversion, and also reduced lesions produced by these mycotoxins.

Compound 12 Summary

> Premium modified clay for broad spectrum
> *Very Effective against*
  > Aflatoxin
  > Fumonisin
  > Ochratoxin
  > Zearalenone
> *Strong Effect against*
  > T-2 Toxin and other tricothecenes; highest neutralizing of products tested to date
> *Not effective against*
  > DON (Vomitoxin); but **neither are any others**

Poultry Dosage (lbs/ton) According to Risk Level

Poultry	Risk Category		
	Low	Moderate	High
Ducklings	1.0	2.0	3.0
Young Turkey Poults	1.0	1.5	3.0
PreStarter-Str Broiler & Turkey	1.0	1.5	3.0
Gro-Fin, Broiler, Turkey, Duck	0	1.0	1.5
Breeder Developer	1	1.5	2.0
Layer, Breeder	1.0	2.0	3.0
Breeder: Broiler, Turkey, Duck, Goose, Quail, Pheasant	1.0	2.0	3.0

Compound 12

Quality Control

Compound 12 Finished Product Testing

> **Mycotoxin adsorption – minimum binding every lot**
  > **Aflatoxin 90%** **- Ochratoxin 90%**
  > **Zearalenone 95%** **- Fumonisin 90%**
> **Heavy metals – lead (<10 ppm), arsenic (<3 ppm), cadmium (<2 ppm), mercury (<1 ppm)**
> **Dioxins – less than 1 part per trillion**
> **Microbiology contamination – less than 1000 cfu/g**
> **Moisture – less than 10%**
> **Texture - >90% pass a 200 micron screen**

Conclusions

> Molds and toxins are a natural hazard and can be costly to game bird enterprises
> T-2 toxin & cousins, Aflatoxin & Ochratoxin are most problematic for game birds
> Work with feed supplier to reduce risk
  > Assure awareness and monitoring of risks
  > Utilize good sampling, analytical capabilities & expertise
  > Manage ingredient usage to reduce risk and prevent effects in sensitive classes of birds
> Utilize other special ingredients as situation dictates
  > Adsorbing ingredients
  > Immune enhancing glucans
  > Anti-oxidant nutrients