

EFFECT OF MYCOTOXIN ON POULTRY PRODUCTION

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Poultry statistics

- Poultry population in India 729.21 millions
- 2.75 million tonnes chicken meat and 65.48 million of hen egg/year
- Per capita availability of egg is 54 pieces (2016-17) against ICMR recommendation 180 pieces and targeted to 110 pieces in 2050
- Per capita availability of meat 2.2 kg (2016-17) against ICMR recommendation 10 kg and targeted to 8.4 kg in 2050
- India produces 5% of global egg production with an annual growth rate 5-8%
- 87.4% of meat comes from chicken meat
- India ranks 3rd in egg production and 5th in chicken meat production

Poultry statistics

- Poultry population in W.B. is 8.62 million in 2012
- Demand of egg is 2.75-2.80 crore eggs/day
- Production 1.8 crore/day
- Shortfall is 1 crore which we buy from other state
- % of share of egg production in 2016-17 are W.B-7.4%, TN-18.9%, AP-18.0%, Telangana-13.4 %
- State wise-per capita availability are W.B.-68, AP-312, TN-237 with the national average- 69 in 2016-17

Medieval History

- The Beggars (1568) painted by Brueghel clearly demonstrated Ergotism
- The victim's limbs, fingers, toes and nose were "consumed by the holy fire that blackened like charcoal"
- Ergotism characterized by convulsions, muscle spasms, hallucinations
- A problem with poor families who consumed rye bread contaminated with ergot alkaloids, originated by the *Claviceps purpurea* fungus.



History

- Aflatoxin was first discovered around 1960 as Turkey X disease in Great Britain which was *A. flavus toxin*
- First outbreak of mycotoxin occurred in poultry farms of Mysore and other parts of Karnataka with a sudden death of 2219 poultry bird (Gopal *et al.* 1969)
- Subsequent report on drop in egg production from 85% to 40% in Warangal in Andhra Pradesh (Sastry *et al.* 1965)
- *Post mortem examination of dead birds* revealed liver lesion with 600 ppb aflatoxin (Choudary 1986)

History

- First human outbreak of mycotoxin was reported from Banswada district of Rajasthan and Panchmahal district of Gujrat
- symptom of hepatitis and death of 106 people was recorded from consumption of 2000-6000 ppb mycotoxin contaminated feed (Krishnamachari *et al.*1975)
- *First correlation between aflatoxin* contamination and hepatomegaly has been report among the children of Canara district of Karnataka (Sreenivasmurthy 1977)

Different types of Mycotoxin

Name	Source	Types	Target organ	Harmful effect
Aflatoxin	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Naturally occurring aflatoxin contains B1, B2, G1, G2. Among them B1 is found in highest concentration and also most toxic.	*Liver *Kidney *Spleen *Testes *Thymus	*Loss of egg production *Anaemia *Haemorrhage *Liver damage *Paralysis *Poor F.C.R *Immunosuppression
Ochratoxin	<i>Aspergillus Ochraceus</i> <i>Penicillium veridicatum</i>	Ochratoxins are of 4 types A, B, C and D. Of these Ochratoxins A is the most toxic.	*Kidney *Liver *Thymus	*Gout *Immunosuppression *Less weight gain *Less egg production and egg weight.
Trichothecene (T2-toxin)	<i>Fusarium spp.</i>			*Reduced feed intake. *Decreased growth. *Immunosuppression.
Citrinin	<i>Penicillium spp.</i>	-----	Kidney	*Wet droppings *Reduced weight gain.
Oosporein			Kidney	*Gout *High mortality

Different types of Mycotoxin

Name	Source	Types	Target organ	Harmful effect
Moniliormin.	<i>Fusarium moniliforme.</i>			<ul style="list-style-type: none"> *Feed intake *Reduced body weight *In chicks it affect heart and cause death *In Layers ,reduced rate of laying and delays peak production.
Fumonisin	<i>Fusarium moniliforme</i>			<ul style="list-style-type: none"> *They are more harmful to pig and horse. *Poultry are more resistant.
Zearalenone				
Ergotism				<ul style="list-style-type: none"> *Feed intake *Growth *Death of tissue of beak,comb and toes. *Diarrhoea *Vesicle and crusts on the comb and wattle. *Less egg production
Fusarochromane				<ul style="list-style-type: none"> * Leg deformities in chicks.

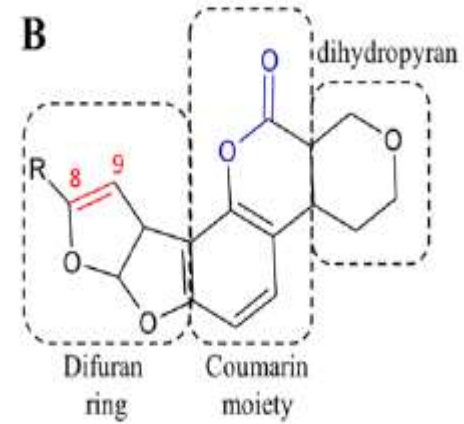
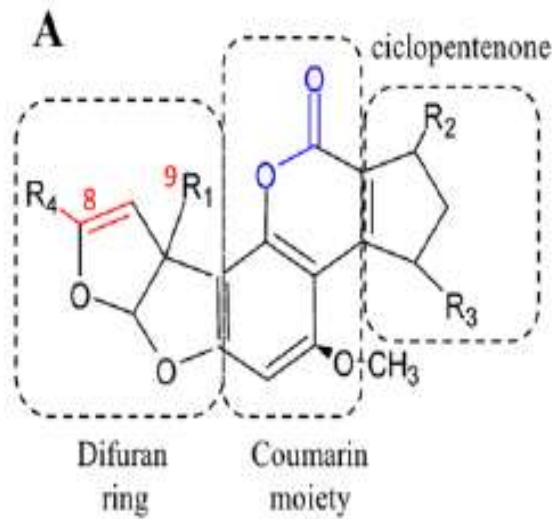
Aflatoxins

- The term aflatoxins (AFs) includes more than 20 fungal secondary metabolites produced by fungi belonging to *Aspergillus* genus
- They are classified into two main groups according to their chemical structure
- The **difurocoumaro cyclopentenone** group includes AFB1, aflatoxin B2 and AFM1, aflatoxin M2
- The **difurocoumarolactone** group comprises aflatoxin G1, aflatoxin G2 and aflatoxin G2a

Aflatoxins

difurocoumaro cyclopentenone

difurocoumarolactone



Aflatoxin

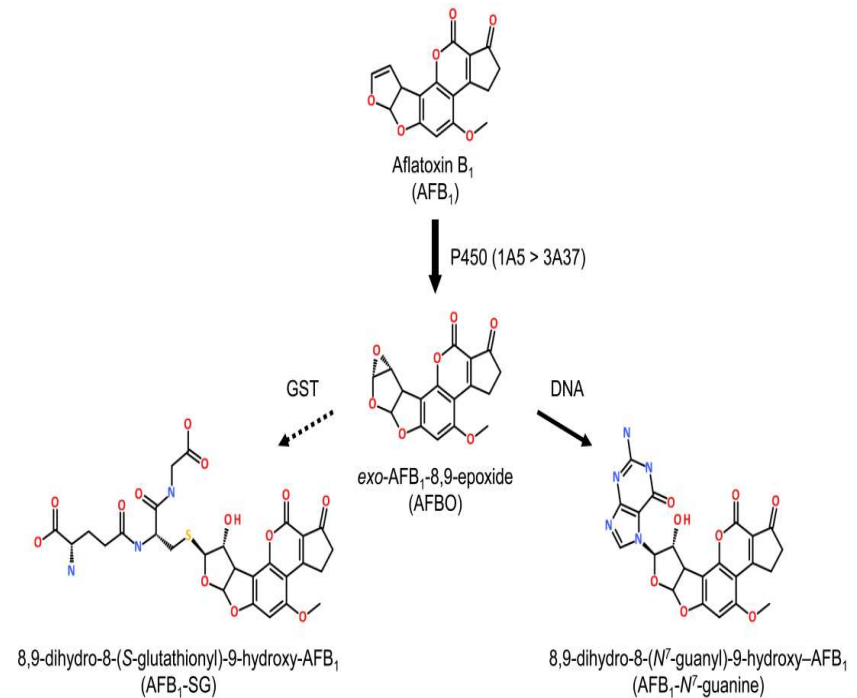
- AFs are difuranocoumarin derivatives composed by two furan rings, linked together to a coumarin moiety
- The furofuran ring has been recognized as responsible for the toxic and carcinogenic activity upon metabolic activation of the C8-C9 double bond to 8–9 epoxide
- The epoxidation is a crucial reaction for AFs carcinogenicity, since it allows the binding to N7-guanine and the subsequent G to T transversions in the DNA
- Lactone ring plays a role in AFs toxicity and carcinogenicity

Deactivating enzyme degrades AFs

- **AF oxidase enzyme** (AFO) isolated from *Armillareilla tabescens* completely degrades AFs at 28°C by enzymatic cleavage of bisfuran ring
- **Manganese dependent peroxidase (MnPs)** from white rot fungi reduces by 90% in 48 hours
- Oxidases from *Myxobacteria fulvus* (**MADE**) degrades the Afs by 98% after 48 hours of incubation
- **Laccases** is isolated from *Peniophora ostreatus* degrades Zearalenone and aflatoxin upto 90%

Metabolism of Aflatoxin

- Metabolize to AFBO by liver CYP-450
- causes
- transversion mutation from G to T in DNA

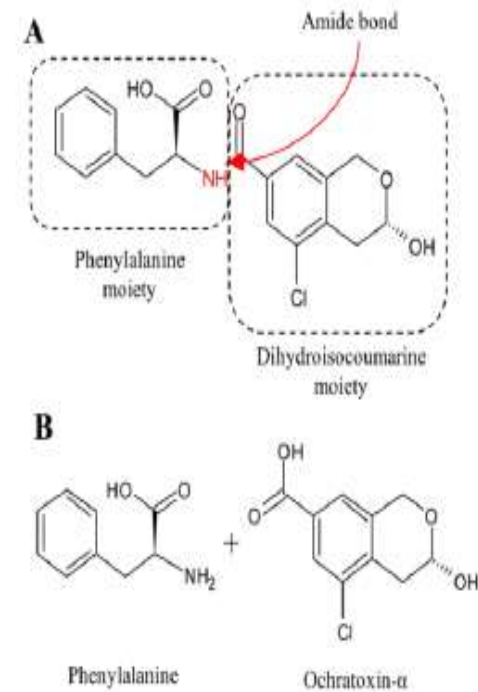


Ochratoxin A

- OTA is a phenylalanine-dihydroisocoumarine derivative linked at the 7-carboxy group by an amide bond
- Due to its structural analogy to the Phe, the toxin can competitively inhibit tRNA phenylalanine synthetases and block protein synthesis
- The main OTA detoxification pathway consists in the hydrolysis of the amide bond between the isocoumarin residue and phenylalanine, resulting in the formation of Phe and OT- α
- The former is considered to be a non-toxic compound, with a 10-times shorter elimination half-life than OTA

Ochratoxin A

- Carboxypeptidase A (CPA) and Carboxypeptidase Y (CPY) hydrolyse the amide bond OT- α
- Lipase from *Aspergillus niger* hydrolyzes the OTA
- Different protease and amidase also hydrolyses OTA into Phe and OT- α

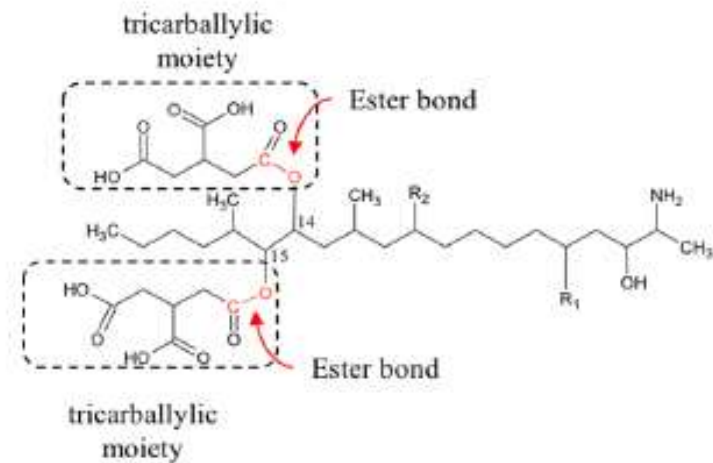


Fumonisin

- Fumonisin are diesters of propane-1,2,3-tricarboxylic acid and similar long-chain amino-polyol backbones
- Structurally they resemble to sphingosine (SO), with tricarboxylic acid groups added at the C14 and C15 positions
- It was described to act by disturbing the sphingolipids metabolism, by inhibiting the enzyme **ceramide synthase**
- Accumulation of sphinganine in cells and tissues

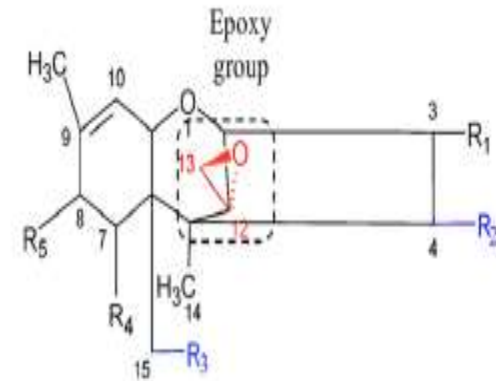
Fumonisin

- 28 different forms of Fumonisin, out of which B-series (FB1) are most abundant and important for toxicity aspect
- Fumonisin is hydrolyzed by *Sphingomonas sp*



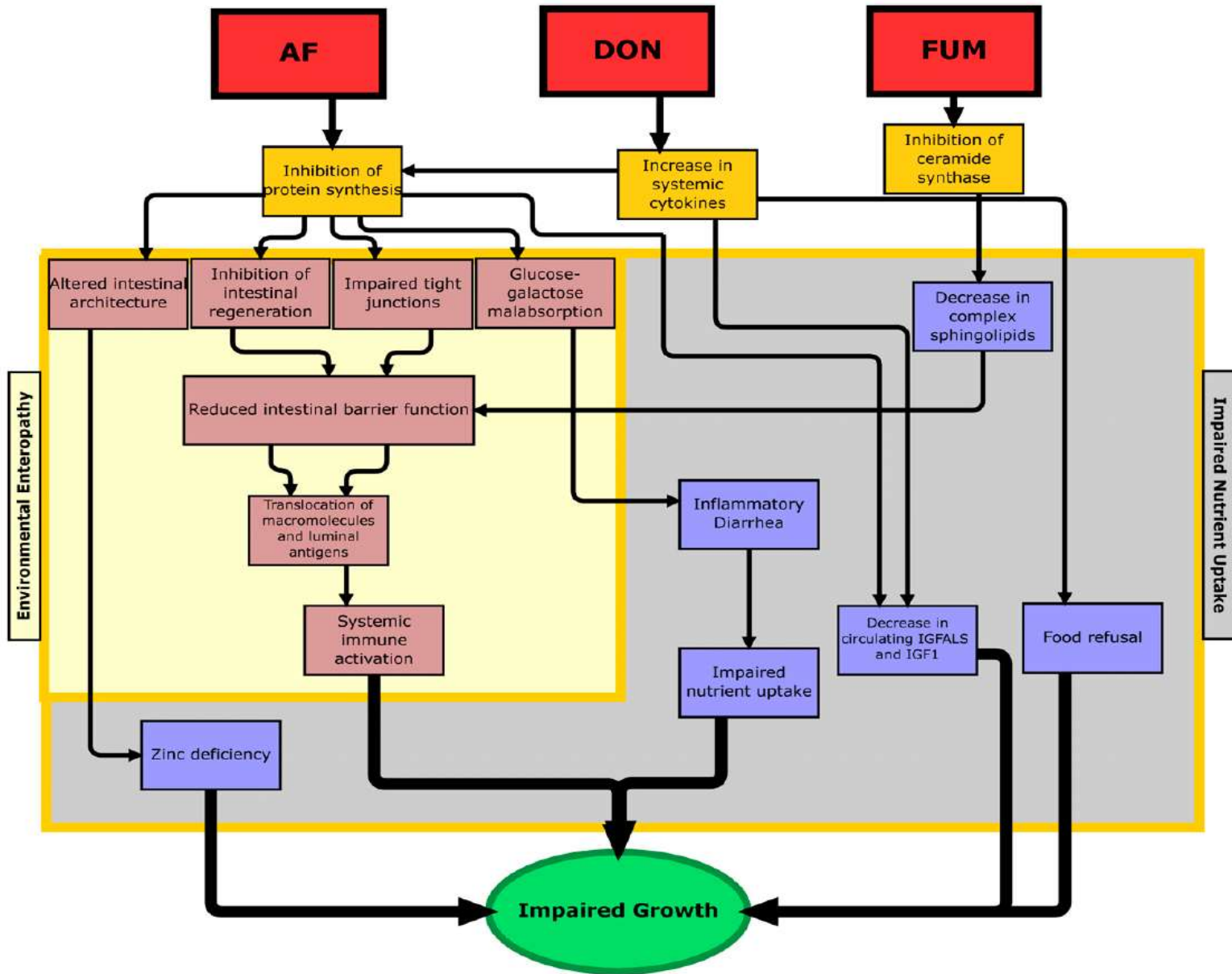
Trichothecenes

- A large group of sesquiterpenoid composed of rigid tetra cyclic ring
- Synthesize from farnesyl pyrophosphates
- Four group, Type-A(T-2, HT-2) and Type-B deoxynivalenol(DON) and nivalenol(NIV)



Trichothecenes

- Type-A is more toxic than other i.e.T-2, HT-2
- T-2 rapidly metabolized in HT-2 in the gut
- Toxin are not degraded during normal poultry feed preparation
- Bacterial cytochrome system P450 from *Sphingomonas* degrades the DON and NIV
- Commercially developed patented product BBSH-797 (Biomin) extracted from *Eubacterium* of rumen fluid degrades T-2, HT-2, DON, NIV



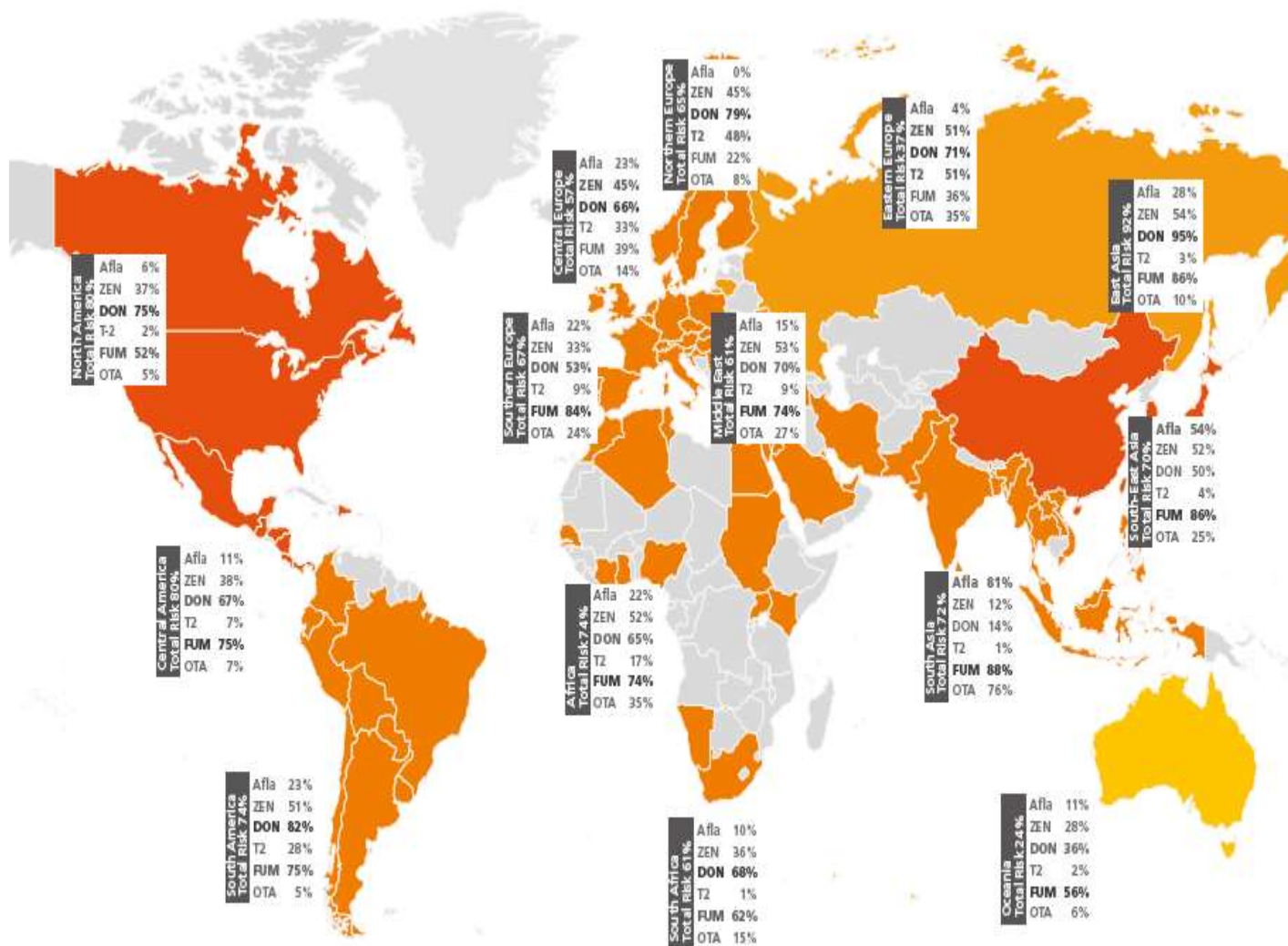


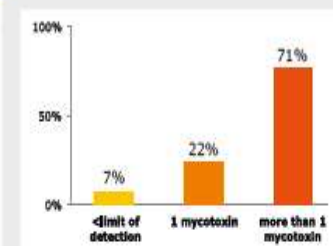
Figure 1. Global map of mycotoxin occurrence and risk in different regions.

Legend

- Moderate risk: 0-25% of samples above risk threshold
- High risk: 26-50% of samples above risk threshold
- Severe risk: 51-75% of samples above risk threshold
- Extreme risk: 76-100% of samples above risk threshold
- No samples tested



Co-contamination



Number of mycotoxins per sample based on samples tested for 3 or more mycotoxins.

Risk Level

The risk level expresses the percentage of samples testing positive for at least one mycotoxin above the threshold level in parts per billion (ppb). A severe risk level indicates that >50% of samples may represent a risk to productivity or disease susceptibility.

Recommended risk threshold of major mycotoxins in ppb

Mycotoxin	Afla	ZEN	DON	T-2	FUM	OTA
Threshold (ppb)	2	50	150	50	500	10

DISCLAIMER
 BDMN GmbH and the authors had no influence on the sampling process of the investigated samples. Therefore, the contamination levels found in the samples do not necessarily reflect the actual contamination level of these regions/commodities. However, the samples provide more insight into the range and levels of mycotoxins which can be found in diverse commodities of various regions.
 MycoSafe is not available in the US and Canada.

ACKNOWLEDGEMENTS
 Special thanks go to Biofarma Feedlab, Argentina, IAMIC, Brazil and Dr. Arlita Stalmhoff-Oester, Tiergesundheitsdienst Bayern e.V. for sharing their mycotoxin analysis results as part of this survey. Mycotoxin Report is published by BDMN Holding GmbH, Eber Campus, 3131 Götzesdorf, Austria, Tel: +43 2782 8030, www.blomn.net

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Mycotoxin contamination throughout Asia

ASIA



Species risk assessment & percentage positive samples

Incidence of mycotoxin in India

- Contamination of mycotoxin in different poultry feed ingredients reported from maize(68%- AF, 26%-OTA,4.0%-ZL, 16%-T-2 toxin)
- Also recorded from broiler feed(AF-20%, OTA-8.6%,2.9%-ZL, 20%-T-2Toxin) from commercial poultry farm of Warangal Telangana (Parvathi D *et al.*, 2017)
- Detection of aflatoxin above 4 ppb (55.56%) reported from poultry and duck feed from West Bengal by LFICA and HPLC (Chowdhury Sumit *et al.*, 2018)
- Detection of aflatoxin by ELISA from maize sample reported (47% in kharif crops) and 17% (rabi crop) in Lucknow (Chandra *et al.*, 2013)

Incidence of mycotoxin

- Presence of residual aflatoxin has been recorded in the highest concentration in liver (2.12 ppb)
- lowest concentration in the breast muscle (0.63 ppb) in broiler
- Birds were fed contaminated feed with 965.12 ppb aflatoxin
- In another study it was reported that eggs (0.66 ppb) fed from 894.12 ppb contaminated feed by HPLC method (Saqer *et al.* 2013).

Younger birds more affected

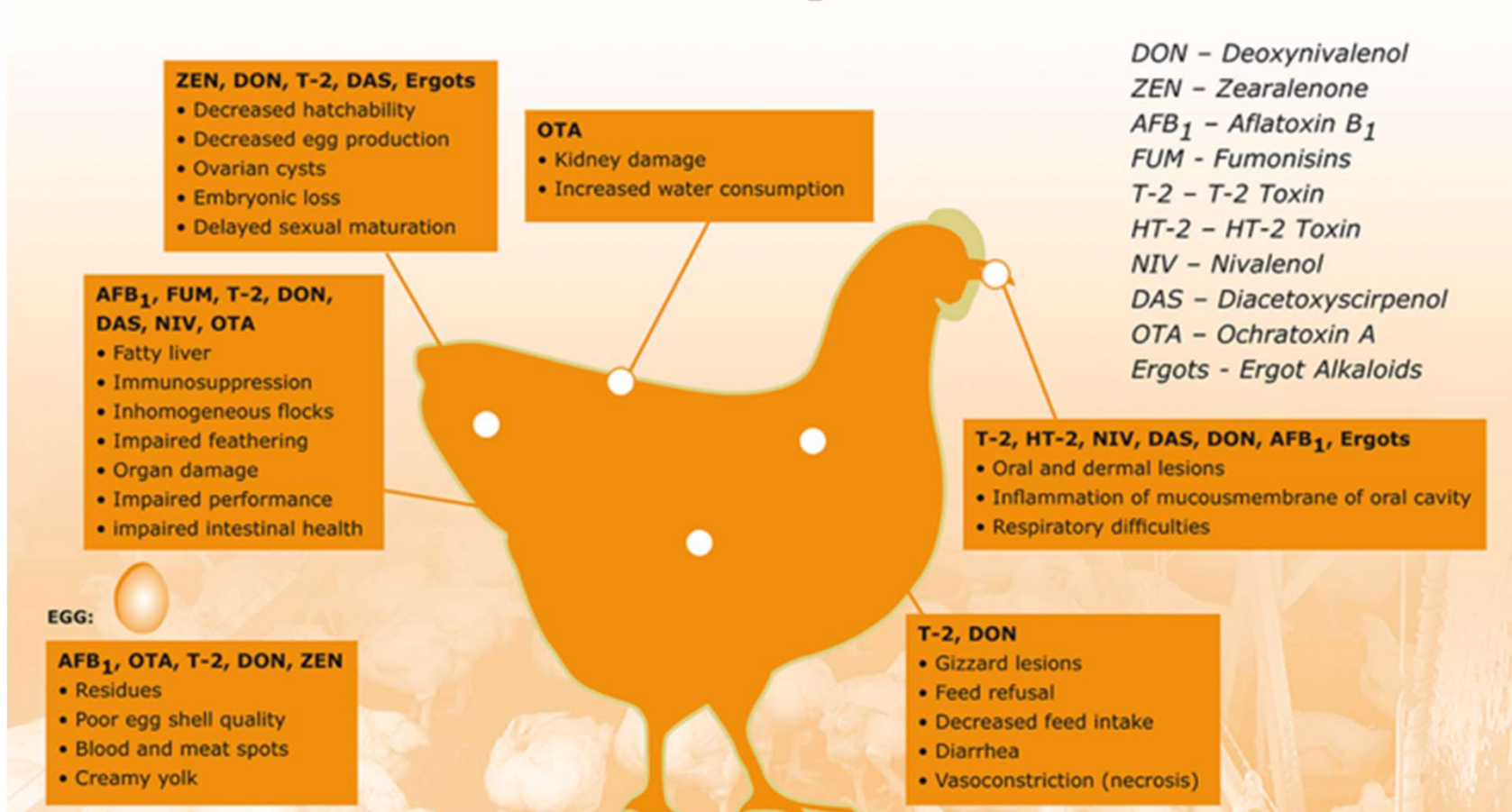
If mycotoxins were present:

- Feed intake 12% ↓
- Body weight 14% ↓
 - Ochratoxins and aflatoxins most severe
- Mortality ↑
 - DON - 8.8 x greater
 - Aflatoxins - 2.8 x greater

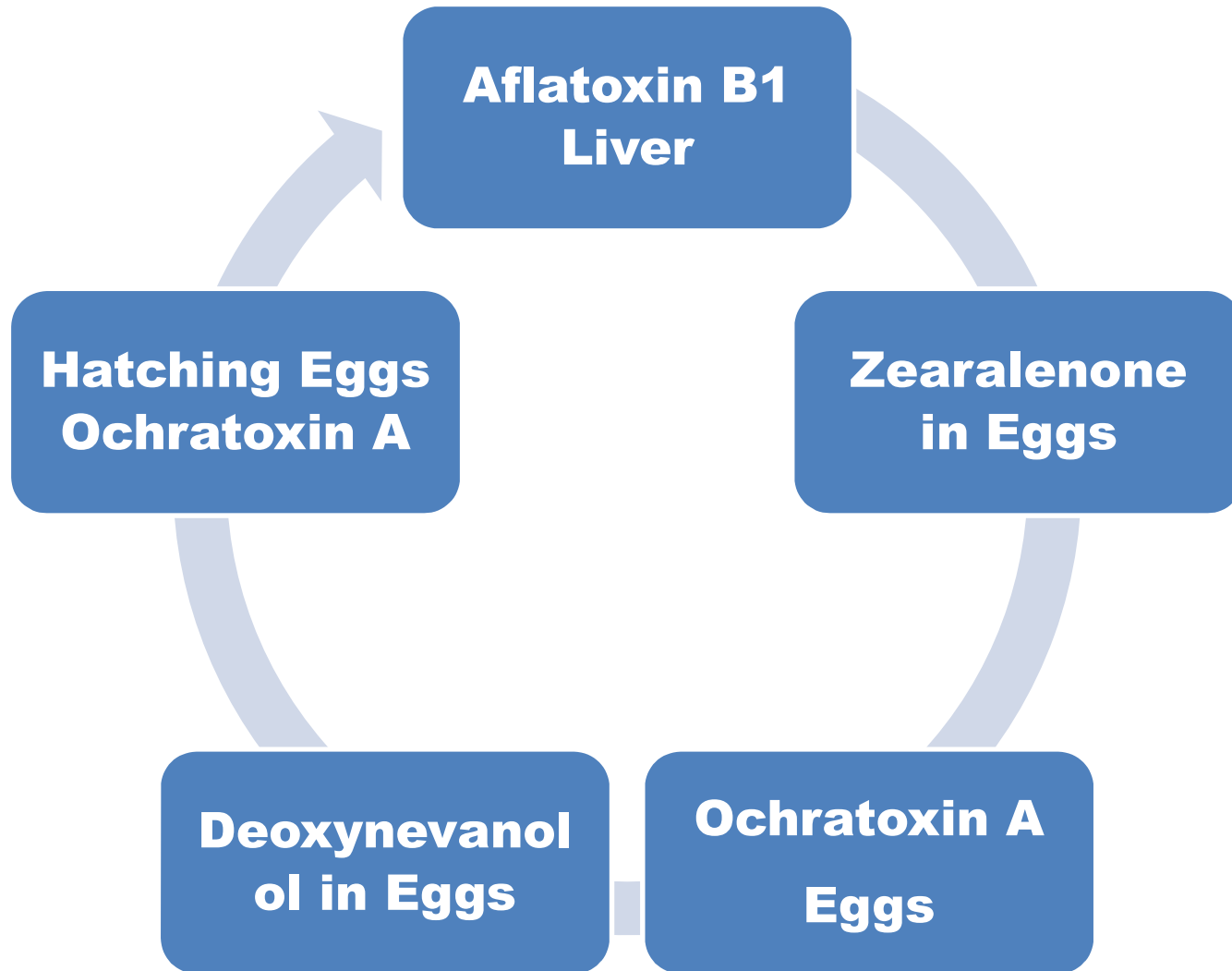
- Organ weights ↑
 - Liver 15%
 - Kidneys 11%
 - Lungs 9%
 - Gizzard 3%



Effects of Mycotoxins



Mycotoxins in Poultry products



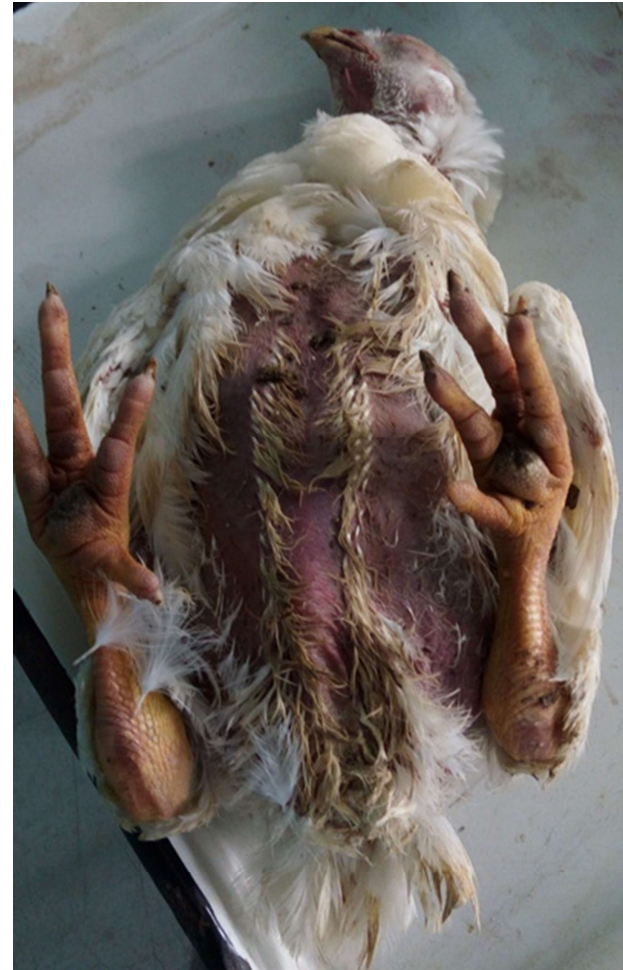
Effect AF on poultry health

- Increased age of maturity in layer, reduced egg production, Egg quality parameter is affected (weight, yolk or albumin %, shell thickness)
- Impair metabolic function of liver
- Avian immune system (bursa, thymus and spleen) depressed
- Both CD4+, CD8+ lymphocyte reduced, Antibody titre of IgG, IgA and IgM reduced
- Decreased the uptake of nutrient as it causes damage of crypt depth of jejunum and villus height in duodenum
- Transferred into egg, reduced hatchability, defects in tibial growth plate

Diagnosis

- Symptom
- Post Mortem Findings
- Laboratory confirmation
 - a. Immuno-chromatographic Assay
 - a. Thin Layer Chromatography (TLC)
 - b. ELISA
 - c. HPLC

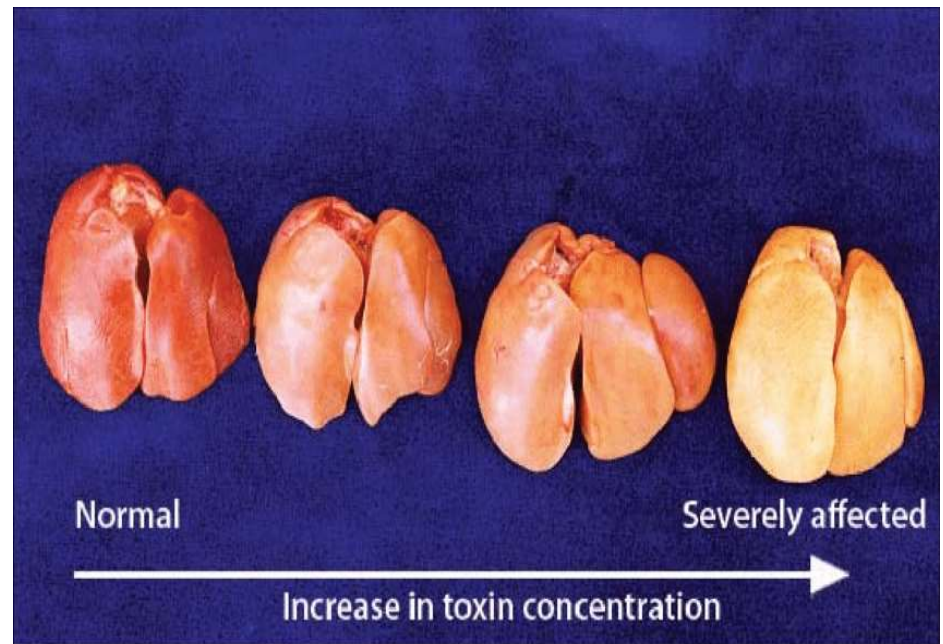
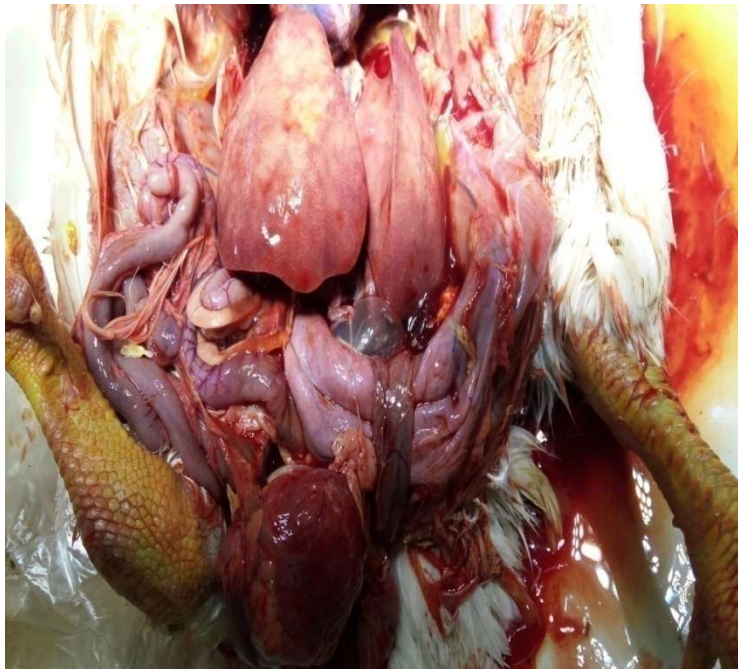
Dead broiler birds



PM of broiler bird



Liver lesion



Lateral Flow Immuno-chromatographic Assay

Sample Preparation:

- Feed samples from different sources are grinded so that 75% would pass through 20-mesh screen
- 10 g of grinded samples were mixed with 20 ml of 70% methanol extraction solution (70/30 (v/v, Methanol/Water))
- The mixture vigorously shaken and vortexed for 1 min
- Samples were allowed to settle, filter the top layer of the extract through Whatman filter paper-1
- The filter was collected subjected to lateral flow immune-diffusion assay.

Lateral Flow Immuno-chromatographic Assay

- Performed by Agrastrip Total Aflatoxin Test, 4 ppb cut-off
- 50 μl of coating conjugates charged in the micro well and 50 μl of test sample charged on to the each well
- One test strip put in each well, allowed to develop color for 5 min
- Two lines visible in test line and control line considered as negative
- A sharp line in the control zone and very faint line in the test zone indicated weak positive sample range from 1- 4 ppb
- A sharp line in the control zone and no visible line in test zone indicated positive sample \geq 1-4 ppb

HPLC analysis

- HPLC system, 1525 pump, 2487 fluorescence detector,
- rp C18 column, guard column (Water make) were used for HPLC analysis
- HPLC graded methanol, HPLC graded-water and trifluoroacetic acid, aflatoxin standard
- Mobile phase methanol: water (45: 55) in 0.5 % trifluoroacetic acid passed through vacuum filter (0.2 μm , 47 mm diameter) for degassing
- Standard was prepared in a final concentration of 1000 ppb in HPLC graded methanol.

HPLC Lab, IAH&VB

Immuno-chromatographic Assay



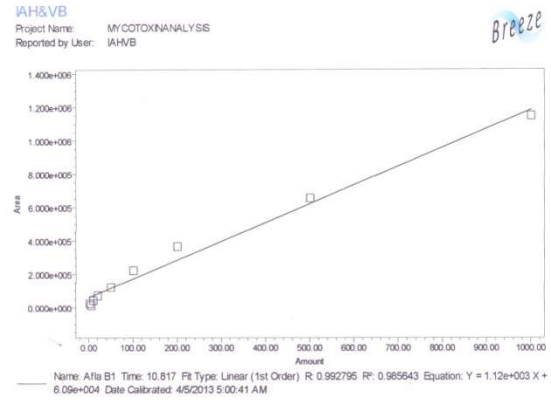
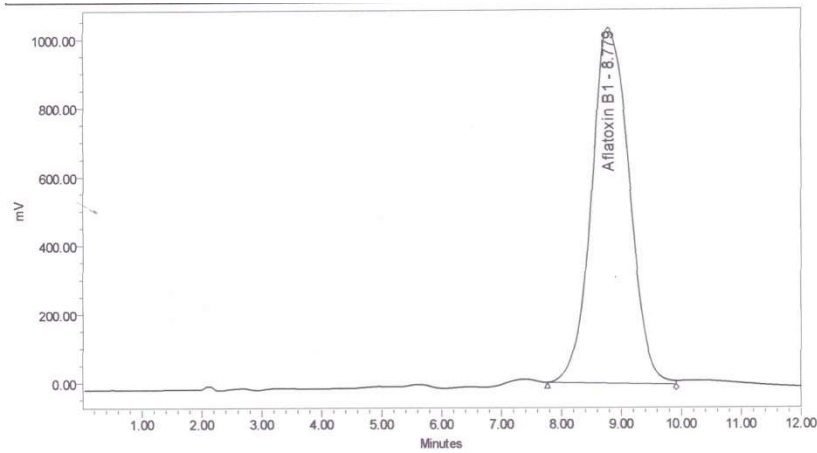
HPLC System



Aflatoxin Analysis in HPLC

Standard curve (B1)

Retention time (Rt)



Peak: Afla B1

Sample Name	Peak Name	Level	Amount	Response	Calc. Amount	% Deviation	Manual Point	Ignore Point
1 Aflatoxin B1 2 ppb	Afla B1		2.000	2.499e+004	-32.002585	-1700.129	No	No
2 Aflatoxin B1 5ppb	Afla B1		5.000	1.348e+004	-42.253227	-945.065	No	No
3					-15.591515	-255.915	No	No
4 Aflatoxin B1 20 ppb	Afla B1		20.000	7.194e+004	9.818027	-50.910	No	No
5 Aflatoxin B1 50 ppb	Afla B1		50.000	1.223e+005	54.708294	9.417	No	No
6 Aflatoxin B1 100 ppb	Afla B1		100.000	2.227e+005	144.116683	44.117	No	No
7 Aflatoxin B1 200 ppb	Afla B1		200.000	3.666e+005	272.284313	36.142	No	No
8 Aflatoxin B1 500 ppb	Afla B1		500.000	6.543e+005	529.520259	5.704	No	No
9 Aflatoxin B1 1 ppm	Afla B1		1000.000	1.147e+006	987.399751	-3.260	No	No

Analysis of feed in 2016-17, IAH&VB

Source	Type of samples	No. of samples	LFICA	HPLC (ppb)
Duck Composite Farm, Burdwan	Grower & Layer duck mash feed	03	>4 ppb	16.00
State Poultry Farm, Purulia	Duck Feed	01	≤ 4 ppb	NA
State Poultry Farm, Malda	Poultry Feed	01	≤ 4 ppb	NA
Private Duck Farm, Sainkrail, Jhargram	Maize & Soya bean	02	≤ 4 ppb	NA
Amrit Feed Hatcheries, Bankura	Poultry Feed	05	≤ 4 ppb	NA

Analysis of Feed sample in 2017-18

Source	Type of samples	No. of samples	LFICA	HPLC
Balliguri Poultry Farm	Poultry Feed	01	>4 ppb	12.25
Amrit Feed Hatcheries, Bankura	Breeder Feed	01	>4 ppb	8.25
State Duck & Poultry Farm, Paschim Medinipur	Poultry Feed	01	>4 ppb	9.25
State Poultry Farm, Cooch Behar	Poultry Layer Mash	02	≤ 4 ppb	NA
State Poultry Farm, Tollygunge	Grower Chick Mash	01	>4 ppb	6.25
Poultry Farm, Haringhata Farm	Layer Feed	01	≤ 4 ppb	NA

Analysis of Feed sample in 2018-19

Source	Type of samples	No. of samples	Observation	Permissible limit (ppb) BIS
State Poultry Farm, Malda	Poultry Feed	01	≤ 4 ppb	NA
Private Poultry Farm, Tajpur, Egra	Starter Feed	02	>4 ppb	8.25
State Poultry Farm, Bishunupur, Bankura	Layer Feed	01	≤ 4 ppb	NA
State Poultry Farm, Kakweep	Poultry Layer Mash	04	≤ 4 ppb	NA
DI, ARD, IAH&VB	Gizzard material	04	≤ 4 ppb	NA
RKM Ashrama, Narendrapur	Duck Feed	01	>4 ppb	10.25
AD,ARD, North 24 Pgs	Duck Feed sample	01	≤ 4 ppb	NA

Mycotoxin Analysis in Other State (2016-17)

Source	Type of samples	No. of samples	LFICA	HPLC
DD,ARD, State Poultry Farm, Agartala, Tripura	Poultry Feed	01	≤ 4 ppb	NA
Poultry Farm, AHLF&VS, Sikkim	Chick Mash	01	≤ 4 ppb	NA
Duck Breeding Farm, Khapuria, CutDuck Breeding Farm, Khapuria, Cuttack	Starter Feed	01	≤ 4 ppb	NA
Poultry Breeding & Research Farm, Angul, Orissa	Poultry Feed	01	≤ 4 ppb	NA
Duck Breeding Farm, Khapuria, CutDuck Breeding Farm, Khapuria, Cuttack	Starter Feed	01	≤ 4 ppb	NA

Managemental approach

- **Mycotoxin screening:** LFICA, TLC, ELISA, HPLC
- **Moisture or Temperature:** Monitoring and control of moisture prevents fungal growth and mycotoxin growth, grain should be kept below 13% of moisture
- **Aspergillus spp.** Does not produce toxin at a temperature below 5-8 °C
- **Cleaning:** Periodic cleaning of feed handling equipments with 5-10% bleaching solution control the mould growth and destroy the aflatoxin to some extend

Control Strategies

- **Pre-harvest:** Development of resistant crop strains by both breeding and direct genetic modification possible(RNAi targeting **polyketide synthetase gene** in maize which produces mycotoxin from aspergillus fungus)
- Also includes prevention of insect infestation, crop residues and crop rotation, irrigation and soil condition and effective drying and storage of grain
- **Harvest:** Timely harvesting and drying of agricultural products controls the production of mycotoxin
- **Post-harvest:** a) **Decontamination methods**
b) **Amelioration or biological inactivation**

Physical method of decontamination

- **Antimycotic agents** such as sorbic acid and sorbate, propionic acid and proprionate, benzoic acid and benzoates and acetic acid prevent mould growth and interfere mycotoxin production
- Potassium sorbate (0.2%) inhibits the fungal growth
- **Herbs** and spices like cinnamom oil, cloves, garlic have anti-mycotic properties
- Wet milling, malting, drying and oil roasting eliminates mycotoxin effectively

Chemical detoxification

- **Ammoniation:** Treatment with aqueous and gaseous ammonia or ammonium hydroxide with or without heat destroy mycotoxin
- Ammoniation not only detoxify mycotoxin (85-100% reduction), but also inhibits the mould growth
- **Sodium hydroxide:** Warming of grain to 105°C in the presence of 0.5% sodium hydroxide detoxifies various mycotoxin
- **Structural degradation:** Numerous chemicals like acids, bases, aldehydes, bisulfites degrades different types of mycotoxins

Mycotoxin-binding agents

Name	Possible MOA	Remarks
Activated Charcoal (AC)	Binds to different types of mycotoxin on the basis of pore size and surface area	<ul style="list-style-type: none"> •Effective against Aflatoxin B1 (95%), Ochratoxin (91%) • Beneficial effect @ 2% level
Bentonites (Hydrated aluminium silicate)	sorberent with layered crystalline micro-structure with interchangeable cations (Na+, K+, Ca++, Mg++)	<ul style="list-style-type: none"> *Sodium bentonite is more effective than calcium bentonite *Binds to aflatoxin to the extent of 66%
Hydrated sodium calcium aluminosilicate(HSCAS)	Molecular surface saturated with polar structure of various mycotoxin	* 0.5% of HSCAS effective against to aflatoxin and OT
Cholestyramine	Ion-exchange resin to strong affinity to OTA	•Reduce OTA upto 50% in in plasma

Anti-oxidant substances

Name	Possible MOA	Remarks
Ascorbic Acid (Vitamin-C)	Reacts with superoxide, hydroxyl radical and singlet oxygen	•Addition of Vit-C to the diet containing OTA protects laying hens
Phenolic compound (gallic acid, vanillic acid, protocatechuric acid)	Inhibits the growth of fungal growth producing Ochratoxin A	
Vitamin E/Selenium	Helps glutathione peroxidase to form water soluble conjugates of aflatoxin	Chicks counter act the formation of lipid peroxide by OTA and T2 toxin
Vitamin A	Prevents mycotoxin induced damage	•Also have anti-carcinogenic properties and reduces the toxicity of OTA

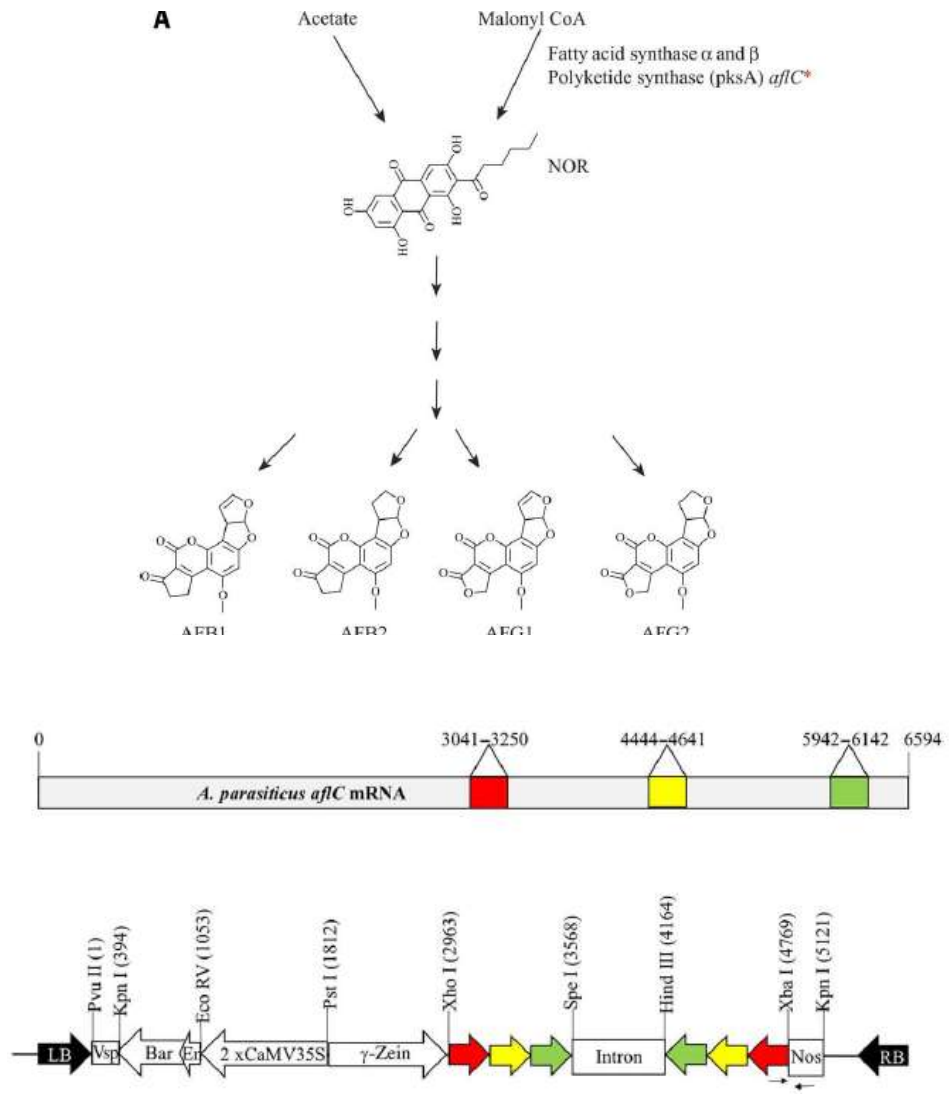
Food components and additives

Name	Remarks
Aspartame	<ul style="list-style-type: none">▪ Structural analogue of OTA▪ Prevents cytotoxic effects of OTA including inhibition of protein synthesis, lipid peroxidation etc.
Crude Protein	<ul style="list-style-type: none">▪ Increase the protein levels from 14-18% to 22-26% counteracts the OT▪ Broiler chick feed with 5 ppm aflatoxin, alleviates the growth and depressing effects of aflatoxins by increasing protein level from 20 to 30%
L-Phenylalanine	<ul style="list-style-type: none">▪ OTA inhibits the protein synthesis by competition with phenylalanine-tRNA synthetase▪ Also inhibits the enzyme phenylalanine hydroxylase which produces tyrosine from phenylalanine▪ Supplementation of 0.8-2.4% of phenylalanine in broiler feed containing 4 ppm OTA decrease the mortality rate by 42.5%
Dietary lipid	Inclusion of cottonseed oil(2-16%)containing 10 ppm aflatoxin improved the body weight by reducing mortality

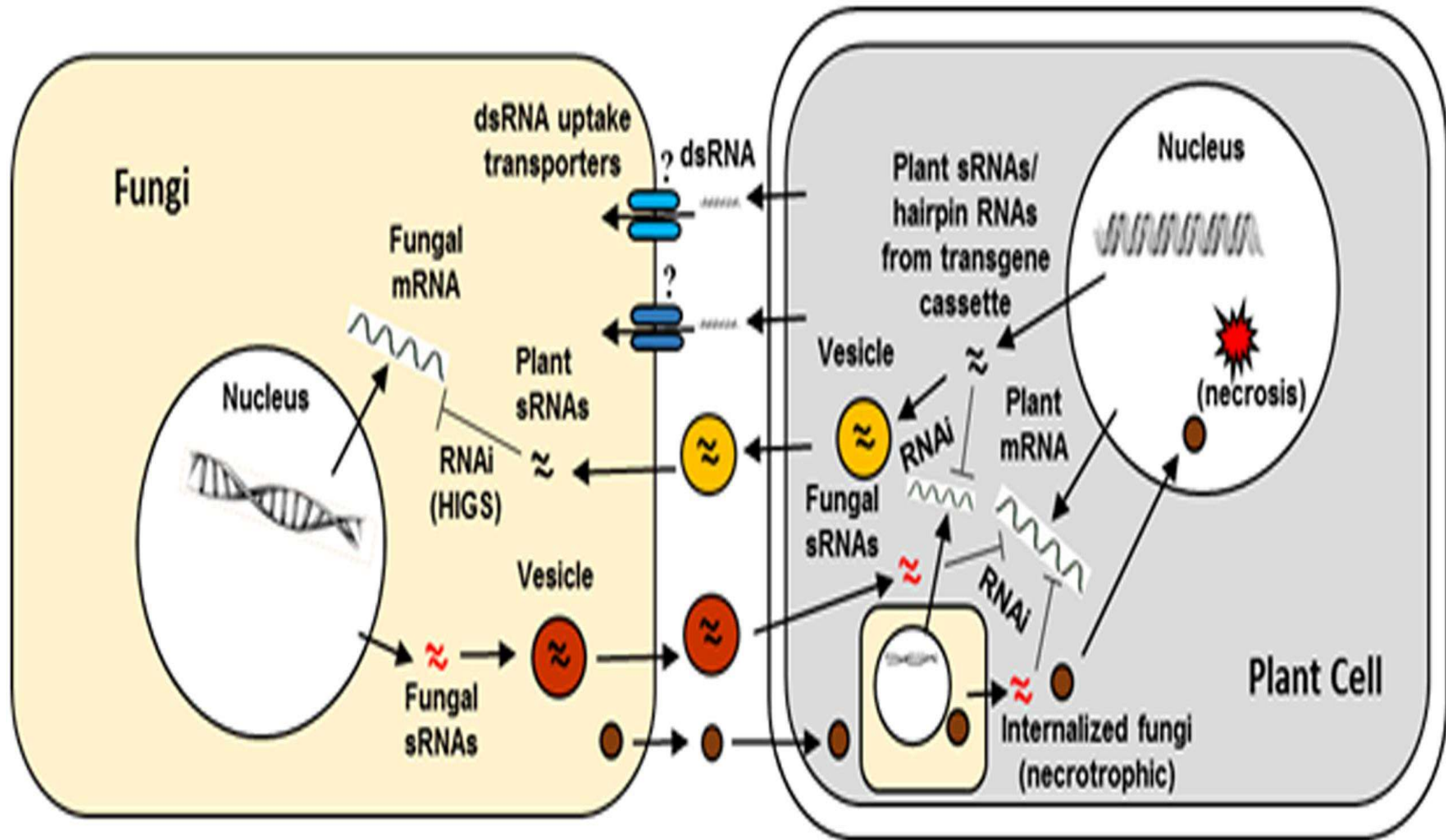
Future road map

- Production of Aflatoxin-free transgenic maize using HIGS
- Future research opportunities of Omics tools
 1. **Metabolomics Approaches:** UHPLC, LC-MS/MS, MALDI-TOF
 2. **Genomics Approach:** RT-qPCR, Microarray Analysis
 3. **Transcriptomics Approaches:** SAGE, WTSS, RT-qPCR
 4. **Proteomics Approaches:** 2D Gel, MALDI-TOF

Aflatoxin free transgenic maize by HIGS

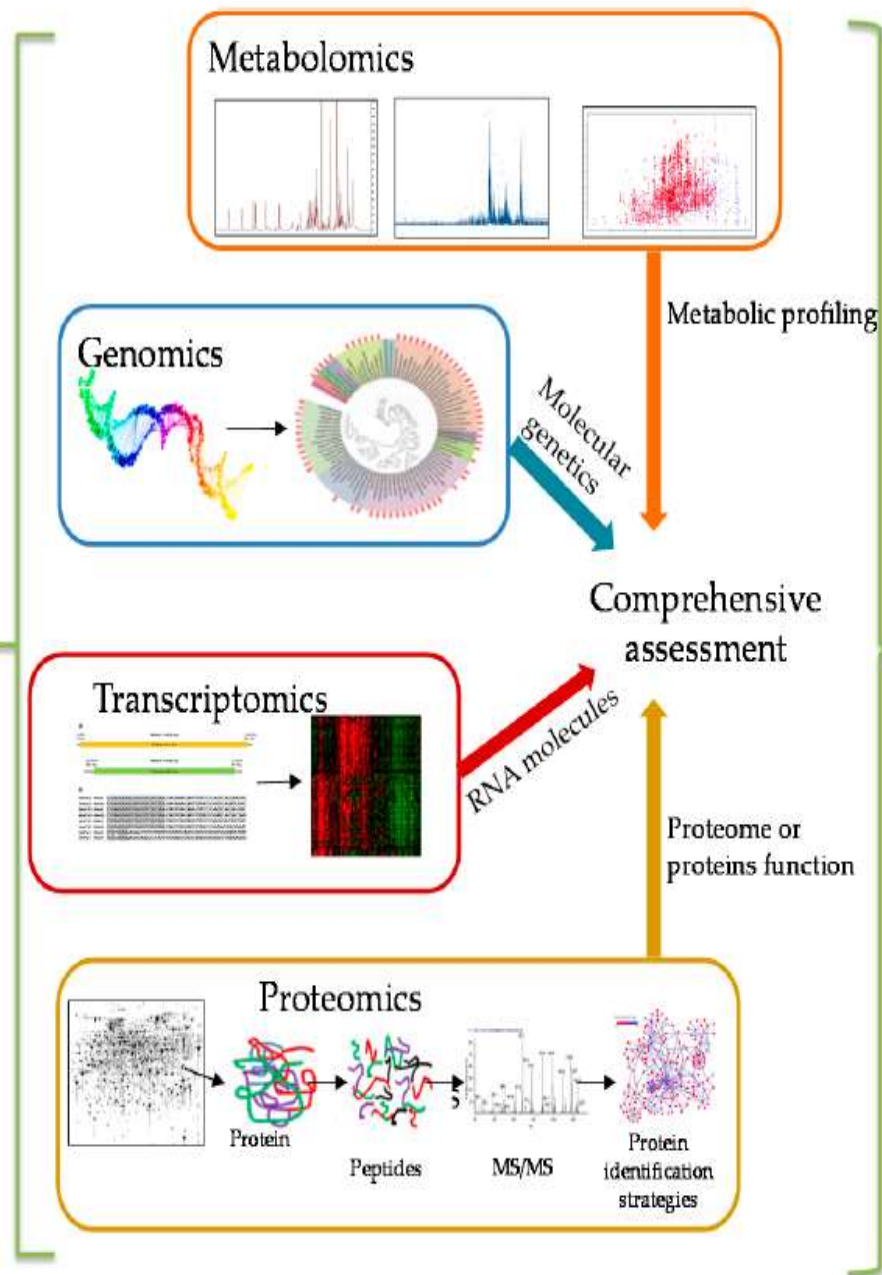


HIGS in Transgenic Maize production





Mycotoxin contamination



Develop an affordable real time solution to mitigate mycotoxin issue pre and post harvesting.

Conclusion

- The problem of mycotoxicosis is not so easy to solve, requires constant attention **throughout the entire process of grain harvest, shipping, storage, feed manufacturing and formulation**
- **Routine analysis of mycotoxin of feed is important step in a control programme in field**
- **Toxicity of mycotoxin can be reduced using mycotoxin-binder, anti-oxidant and food additives in the poultry feed**
- **Production of aflatoxin free transgenic maize by HIGS will reduce the toxicity problem in poultry health**

THANK YOU