CEVA SANTE ANIMALE
Ceva Animal Health Asia Pacific

HATCHERY
EXPERTISE ONLINE
ceva.asiapacific@ceva.com

Issue No.23 / March 2009

MYCOTOXINS AND DAY–OLD CHICK QUALITY

Dr Vincent TURBLIN – Deputy Regional Market Manager – Poultry
CEVA Animal Health Asia Pacific – Selangor, Malaysia

INTRODUCTION

Nowadays, the poultry industry gives a lot of importance to costs management at all the steps of production. In broilers, the production of hatching eggs or Day-Old Chick (DOC) does not represent a high proportion of the overall cost of production per kg of bird slaughtered. Nevertheless, the performance of broiler breeder is looked with a lot of attention, and the number of saleable chicks per hen is always very focused. But the quality of DOC and especially its influence on the improvement of their performance is a bit neglected. In general, the way that parental nutrition impacts the subsequent broiler performance is poorly studied: most of the time, egg fertility and hatchability are more emphasized.

The feed costs for the breeder layer has to be compared with the total feed cost of production of one live harvested broiler : only 310 g of breeder feed is needed per DOC produced (and this cost includes pullet stage), in comparison with the 3.6 kg for growing a DOC into a 2 kg-broiler….

What are the effects of selected nutrients and anti-nutrients like vitamins, micro-minerals and mycotoxins? We will review here only this last point and especially take care to the immune function and carcass yield of the offspring.

DIFFERENT TYPES OF MYCOTOXINS

Mycotoxins are a broad class of chemicals, produced by a very diverse population of molds. Additionally, the chemical structures of mycotoxins differ greatly. Therefore the pathological effects will show big differences: some of them are extremely toxic whereas some are almost non-toxic.

They will have **local** and **systemic impact** mainly on **quickly multiplicative tissues and cells**. Obviously the embryo tissues are edictible target for those compounds.

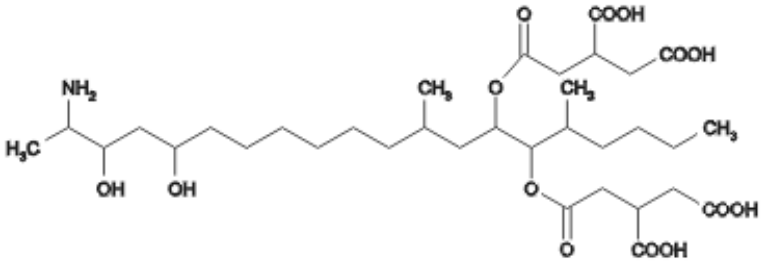


***Figure 1**: Fumonisin B2.*

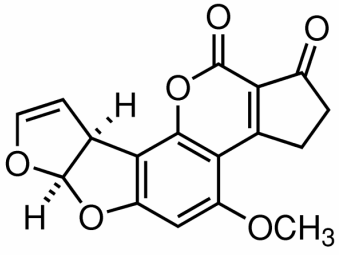


***Figure 2**: Aflatoxin B1*

EFFECTS OF MYCOTOXINS IN BROILER BREEDER FEEDING ON PROGENY

The bibliography is not as rich to describe the repercussions of fungal metabolites or toxic products on progeny health as it is for dietary nutrients. Kidd (2003) had reviewed those ones and the following table shows some major researches on this topic.

Table 1 *- Effects of non nutritional compounds in breeder hen nutrition on progeny performance (adapted from Kidd, 2003)*

Compounds in breeder feed	*Effects on progeny*	*Parameter:*	*Authors*
0, 5, 10 µg/g aflatoxin	No	Mortality, feed efficiency and weight gain at 14 d	Howarth & Wyatt, (1974)
0, 0.2, 1, 5 mg/kg aflatoxin B	Yes	Decreases cellular and humoral immunity	Qhreshi *et al.*, (1998)
0, 10, 25, 50, 100, 200, 400, 800 mg/kg zearalenone	No	Body weight at 0 and 21 days	Allen *et al.*, (1981)
0, 30, 300, 3000 mg/kg insect regulator CGA-72662 (Larvadex®)	Yes	Depressed body weight at 14 dwith high levels in progeny from young dam	Brake *et al.*, (1984)
0, 30, 300, 3000 mg/kg insect regulator CGA-72662 (Larvadex®)	No	Feed efficiency and mortality at 14 days	Brake *et al.*, (1984)
400 mg/kg furazolidone, nitrofurazone, furaltadone, nitrofurantoin	Yes	Residues of nitrofurazone metabolite (semicarbazide) in liver and muscle of day old chicks.	McCracken *et al.* (2005)
400 mg/kg furazolidone, nitrofurazone, furaltadone, nitrofurantoin	No	Residues in chicken at 40 days	McCracken *et al.* (2005)

Even if smaller size of DOC is very often described, Howarth (1974) showed no significant differences at harvesting time. Nevertheless, it is well acknowledged that management of such smaller birds is more difficult and at the end of the day, the birds harvested do not show the same level of performances than bigger size DOC (MEIJERHOF, 2009).

Furthermore, Aflatoxins and Zearalenone were mainly targeted in these researches, and their transfer through in eggs showed impact on the progeny immune function (Qhreshi et al., 1998). Other classes of mycotoxins classically present in poultry feed shouldn't be forgotten nevertheless.

Aflatoxins (B1, B2, G1 and G2)

Among the aflatoxins, the most toxic is B1 and it is also the major one in terms of concentration. *Aspergillus flavus* and *Aspergillus parasiticus* are recognized as the molds responsible for the production of aflatoxins. In most feedstuffs, *A. flavus* tends to produce primarily aflatoxin B1, whereas *A. parasiticus* tends to produce a mixture of both **aflatoxin B1 and G1.**

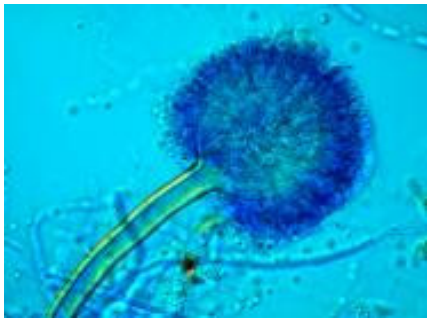
The basic mechanism of action of aflatoxin at the cellular level is to **bind covalently with DNA**, with subsequent inhibition of protein synthesis or disruption of normal regulatory processes. For instance, failure of affected birds to produce digestive enzymes is noted during aflatoxicosis. This process results in incomplete digestion of feed, passage of undigested feed in the feces and poor growth and performance. But even more important, it will impair the development of all multiplication cells: again, embryos and young birds will suffer from this. Ducklings and broilers also, are very susceptible.

Moreover, susceptibility of poultry to aflatoxicosis is dependent upon the presence of interacting factors; age of the birds and species. For example, breed, strain and gender of poultry will influence susceptibility of aflatoxin poisoning. Young broilers are more susceptible to aflatoxin than older broilers. And obviously, even if broiler breeder hen does not respond to aflatoxin in a significant manner, the transfer of aflatoxin residues to hatching eggs can result in high embryonic mortality and impact the growth of DOC later on.

The half-life of aflatoxin B1 in hens is about 67 hours (SAWHNEY, 1973). The feed:egg transmission of aflatoxin B1 is about 5000:1 (OLIVEIRA, 2000). Most aflatoxins are excreted through the bile and intestine of the hens, but aflatoxin B1 and aflatoxicol could be identified *in ova* for 7 days or longer (JACOBSON, 1974 ; TRUCKNESS, 1983). Aflatoxin B1 accumulated in reproductive organs will be transferred to eggs (both yolk and albumen) and hatched progeny (yolk sac and liver) in chickens, turkeys, and ducks (SOVA, 1986).

Embryonic exposure to aflatoxin B1 has been shown to depress graft-versus-host and lymphoproliferation reactions (DIETERT, 1985), and depress several macrophage effector function reactions in post-hatch chick (NELDON-ORTIZ, 1992). Because these former reports have demonstrated that embryonic exposure of aflatoxins depresses chick immunity, Qureshi (1998) evaluated progeny immunity from hens fed 0, 0.2, 1.0 and 5.0 mg/kg aflatoxin. The presence of aflatoxin in the hen's diet, especially of higher levels, decreases cellular (macrophage viability, phagocytosis and reactive oxygen intermediates) and humoral immunity (QURESHI, 1998). The aflatoxin levels have to be monitored in ingredients fed to hens so that the disease fighting ability of progeny is not compromised.

Ochratoxins



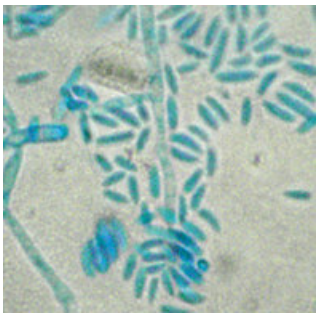
These nephrotoxic metabolites are produced especially by *Penicillium viridicatum* and *Aspergillus ochraceous.* The most toxic is **Ochratoxine A**.

Breeder hens fed with feeds contaminated with ochratoxin showed reductions in body weight, egg production and egg weight (PRIOR, 1981). Besides that, ochratoxin can reduce egg size, interior quality, and shell specific gravity.

Ochratoxin A distributes to egg yolk and albumin (FRYE, 1977), which accounted for reductions in hatchability. In chickens, hatchability is reduced mainly by embryonic mortality due to embryonic gout, and progeny had reduced growth (NIEMEC, 1995). Ochratoxin A was also teratogenic for chicken embryos (GILANI, 1975).

Like other general features of aflatoxins, the immune system can be impaired (thymus atrophy, etc.). And finally, ochratoxins can interfere with osteosynthesis (bone weakness).

Mycotoxins from the Genus Fusarium



Most of these mycotoxins are produced by *Fusarium moniliforme.*

The major concerns related to them in poultry are undoubtedly **trichotecenes (T2)**. For sure, one of the actions is at least to decrease the feed consumption of the breeders (oral and gut lesions), and thus reduce the nutrients transmitted to the progeny (like deficiency in Vitamine E or B1). This effect can result as well from the reduction of cell mitotic activity in intestinal crypts, and malassimilation on breeder flocks. And finally, the immune system being impaired by this toxic compounds, the immune status of the progeny can be in the same way affected: less antibody transmitted, etc (HAQ, 1996 ; HOSSAIN, 1998).

But the direct toxicity on embryo and subsequent DOC is not well documented. Relatively small amounts of T-2 toxin are excreted into the egg (84). Progressively greater amounts of T-2 metabolites are excreted into yolk, but those in egg white peak and remain constant (CHI, 1978).

The mechanism of **fumonisin B1** toxicity is related to disrupted sphingolipid synthesis. But, no residue of fumonisin could be found in the eggs in some experiments involving intravenous injection of this mycotoxin. Nevertheless embryos are quite susceptible to this toxic:

Table 2: *Embryolethal action of FB1 in poultry (JAVED, 1993)*

Age of EMBRYO	CONCENTRATION OF FB1 inoculated *	MORTALITY RATE
1 day	1 µM	50 %
	10 µM	70 %
	100 µM	100 %
10 days	1 µM	30 %
	10 µM	60 %
	100 µM	80 %

** 100µL of solution injected per egg*

The lesions observed are haemorrages, hydrocephalia, beak enlarged (chondrostimulation), pale kidneys, gut impairment and FB2 and FB3 are overall much more toxic than FB1 (HENRY, 2001). Some birds surviving after inoculation won't manage to break the eggshell. On the other hand, there is a strong delay in feather development, and their color is "metallic" or rusty (THIBAULT, 1997).

Furthermore, it is necessary to keep in mind that all these mycotoxins from Fusarium (including deoxynivalenol and zearalenone) can act in synergy with other factors, and endanger DOC quality, even if they have few or no direct toxicity on embryo itself.

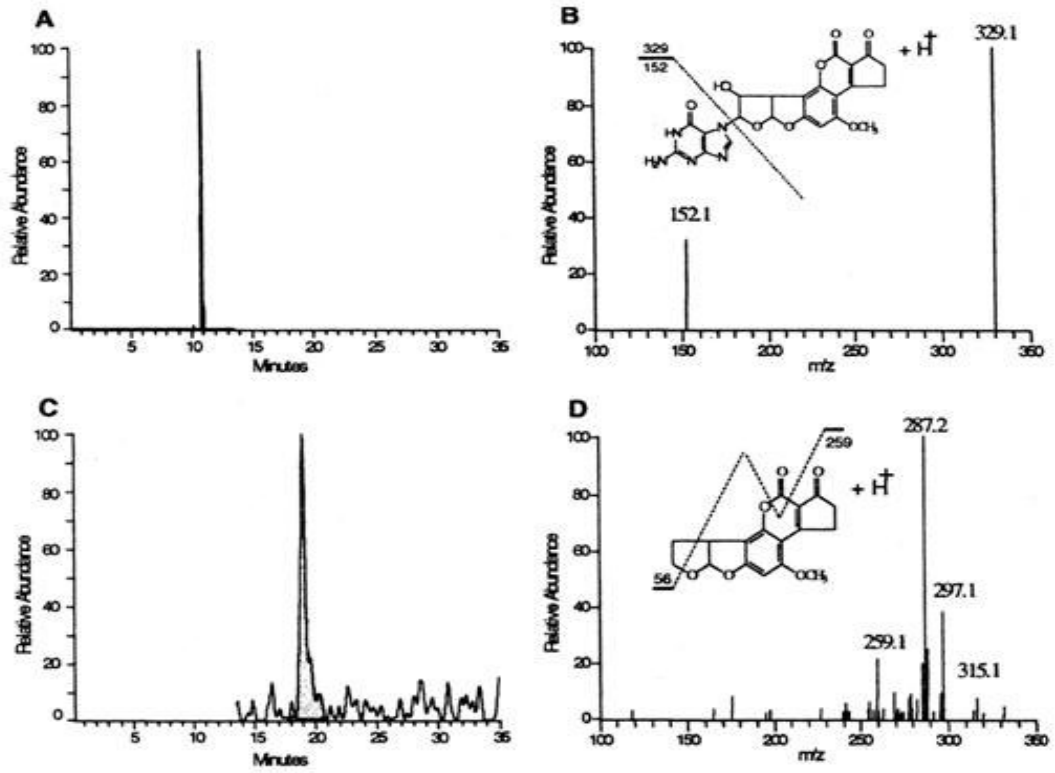
ANALYSIS OF MYCOTOXINS

Tissue analysis of mycotoxins is almost always futile. The underlying reason for this is due to the fact that mycotoxin concentrations at the time the tissue sample is obtained will be extremely low or non-existent. Nevertheless, in experimental conditions it is possible to find measurable quantities of aflatoxin in both egg white and yolks, like Jacobson wrote in 1974.

Once most mycotoxins are consumed, the mycotoxin or its metabolites and adducts are rapidly excreted from the bird and no residue will persist. The most interesting approach to investigate this hypothesis is a chemical analysis of the feed being consumed by the breeders: it is not practically possible.

The specific method of analysis could involve chemical absorbancy, Thin Layer Chromatography (TLC), High Pressure Liquid Chromatography (HPLC) or Gas Chromatography – Mass Spectral methodology (GC-MS). Specific techniques allow numerous mycotoxins to be analyzed. But they are time-consuming, costly, and often require specialized equipment and training of laboratory personnel: therefore, they are more adapted for research purposes.

***Figure 3**: Liquid chromatography-electrospray mass spectrometry of aflatoxin-N7-guanine (DNA-adduct of aflatoxin B1).*



Egner P. A. et.al. PNAS 2001;98:14601-14606

More practically, exploitation of enzyme-linked immunosorbent assay (ELISA) technology has resulted in the development of testing procedures for mycotoxins in a variety of commodities, most of the time in monoclonal antibody-based detection kits. These tests can yield mycotoxin concentrations with ppb accuracy or can be modified to give a less expensive qualitative result. They have proven to be not only highly accurate and specific but also free from most interfering influences. For the particular problem of Aflatoxin, green fluorescence under a black (ultraviolet) light can help to estimate the degree of *A. flavus* contamination.

CONCLUSION

The toxic compounds are not only from molds, but can have other origins also. The effects of synthetic compounds, as those used to control pests in poultry houses, on progeny performance have been investigated (Brake et al.,1984). Feeding hens with diets containing an insect growth regulator can negatively affect broiler chicken performance.

Studying toxic and anti-nutrient is totally linked to the concern of optimizing nutrition of breeders. It is necessary to capitalize on it in order to give the DOC a good stock for starting their growing period.

Furthermore, those researches are complementary to the fast developing **technology of in-ovo injection of drugs and nutrients:** this will undoubtedly make the possibility of influencing broiler performance much more easily than it has been in the past, giving a real and practical tool to the large scale poultry producers.

MYCOTOX® NG

>>> BINDING AND BEYOND >>>

REFERENCES

BRAKE J, ORT JF, CARTER TA, CAMPBELL WR. Effect of the insect growth regulator CGA-72662 (Larvadex®) on broiler breeder production, hatchability and subsequent chick performance. Poultry Science,1984; ***63****: 910-916.*

CHI MS, ROBISON TS, MIROCHA CJ, SWANSON SP, SHIMODA W. Excretion and tissue distribution of radioactivity from tritium-labeled T-2 toxin in chicks. Toxicol Appl Pharmacol, 1978; ***45****: 391-402.*

DIETERT RR, QURESHI MA, NANNA UC, BLOOM SE. Embryonic exposure to aflatoxin B1: mutagenicity and influence on development and immunity. Environmental Mutagenesis, 1985; ***7****: 715-725.*

FRYE CE, CHU FS. Distribution of ochratoxin A in chicken tissues and eggs. J. Food Saf., 1977; ***1****: 147—159.*

HAQ A, BAILEY CA, CHINNAH AD. Effect of beta-carotene, canthaxantin, lutein and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. Poultry Science, 1996; ***75****: 1092-1097.*

HENRY MH, WYATT RD. The toxicity of fumonisin B1, B2 and B3, individually or in combination, in chicken embryos. Poultry Science, 2001; ***80****: 401-407.*

HOSSAIN SM, BARRETO SL, BERTECHINI A, RIOS AM, SILVA CG. Influence of dietary vitamin E level on egg production of broiler breeders, and on the growth and immune response of progeny in comparison with the progeny from eggs injected with vitamin E. Animal Feed Science and Technology, 1998; ***73****: 307-317.*

JACOBSON WC, WISEMAN HG. The transmission of aflatoxin B1 into eggs. Poult. Sci.,1974; ***53****: 1743-1745.*

JAVED T, BENNETT GA, RICHARD JL, DOMBRINK-KURTZMAN MA, COTE LM and BUCK WB. Embryopathic and embryocidal effects of purified fumonisin Bl or Fusarium proliferatum culture material extraction chicken embryos. Mycopathologia, 1993; ***123****: 185-193.*

KIDD MT. A treatise on chicken dam nutrition that impacts on progeny. World's Poultry Science Journal, 2003; ***59****: 475-494.*

MCCRACKEN RJ, VAN RHIJN JA, KENNEDY DG. Transfer of nitrofuran residues from parent broiler breeder chickens to broiler progeny. British Poultry Science, 2005; ***46****: 287-292.*

MEIJERHOF R. The benefits of extra-centimeters. HatchT. Incub. Techno., 2009.

NELDON-ORTIZ DL, QURESHI MA. Effect of AFB1 embryonic exposure on chicken mononuclear phagocytic cell-functions. Develop. and Compar. Immunol., 1992; ***16****: 187-196.*

NIEMIEC J, BORZEMSKA W, ROSZKOWSKI J, KARPINSKA E, KOSOWSKA G,SZELESZCZUK P. Pathological changes in chick embryos from layers given feed contaminated with ochratoxin A. Med Weter, 1995; ***51(9)****: 538-540.*

OLIVEIRA CA, KOBASHIGAWA E, REIS RA, MESTIERI L, ALBUQUERQUE R, CORREA B. Aflatoxin B1 residues in eggs of laying hens fed a diet containing different levels of the mycotoxin. Food Addit. Contam., 2000; ***17****: 459-462.*

QURESHI MA, BRAKE J, HAMILTON PB, HAGLER JR WM, NEISHEIM S. Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicks. Poultry Science, 1998; ***77****: 812-819.*

PRIOR MG, SISODIA CS, O'NEIL JB. Effects of ochratoxin A on egg production, body weight and feed intake in white leghorn hens. Poult Sci, 1981; ***60****: 1145 - 1148.*

SAWHNEY DS, VADEHRA DV, BAKER RC. The metabolism of [14C] aflatoxins in laying hens. Poult. Sci., 1973; ***52****: 1302-1309.*

SOUTHERN LL, BAKER DH, SCHMEISSER DD. Eimeria acervulina infection during aflatoxicosis in the chick. Nutr. Rep. Int., 1984; ***29****: 35-44.*

SOVA Z, FUKAL L, TREFNY D, PROSEK J, SLAMOVA A. B1 aflatoxin (AFB1) transfer from reproductive organs of farm birds into their eggs and hatched young. Conf. Europeenne d'Aviculture, 1986; ***7****: 602-603.*

THIBAULT N, BURGAT V, GUERRE P. Les Fumonisines: nature, origine et toxicite. Revue Med. Vet., 1997; ***148, 5****: 369-388.*

TRUCKSESS MW, STOLOFF L, YOUNG K, WYATT RD, MILLER BL. Aflatoxicol and aflatoxins B1 and M1 in eggs and tissues of laying hens consuming aflatoxin-contaminated feed. Poult. Sci, 1983; ***62****: 2176-2182.*