The impact of mycotoxins on the vaccination of poultry

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Vaccine development is performed under research conditions. In R&D, one strives to reduce factors of variation to increase the chances of showing significant effects of the researched issue. Therefore, vaccine development is performed with excellent quality feeds to avoid variation in feed quality interfering with the effect of the vaccines.

Practical vaccine application is performed under field circumstances. Veterinarians are surely aware of the precautions necessary to avoid vaccine inactivation, thus ensuring optimal functionality in the animals. But, are they equally aware of the need to optimise the animal's capacity to respond to the concerned vaccines?

Are they aware that, in field circumstances, mycotoxins may decimate the response to vaccination?

Mycotoxin history

In 1960, although ubiquitously present, mycotoxins were still unknown; a dramatic disease outbreak in the UK was then named 'Turkey X disease' (since some 100,000 turkeys died in that year).

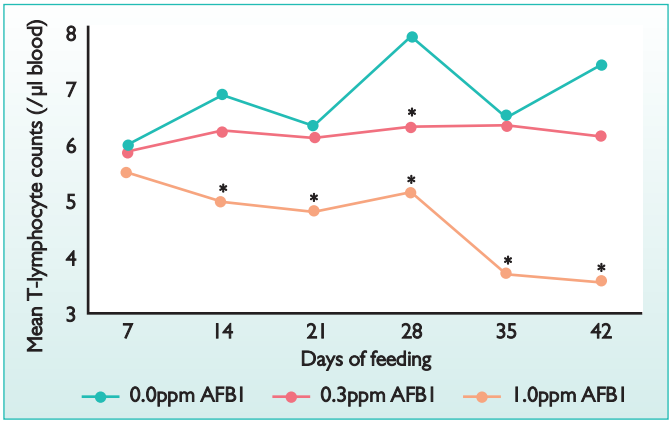

Fig. 1. Effect of feeding purified AFB1 to broiler chickens on mean T-lymphocyte counts (Ghosh et al, 1991).

The disease was linked to the import of a ship load of peanut meal from Brazil; scientists were able to extract and transfer the causative agent.

The same causative agent was found in other feedstuffs such as coconut meal and later on also in corn. It was revealed that Aspergillus flavus produces the chemical, which lead to defining 'aflatoxin' as the first mycotoxin; its chemical nature was elucidated during the mid-sixties.

In 1964 it was found that aflatoxins are not only produced by A. flavus and A. parasiticus, but also by, for example, Penicillium spp.

Nevertheless, the focus remained on mycotoxins produced during storage; many years went by before pre-harvest production of aflatoxin was also recognised as a problem.

Carcinogenicity in rats was already discovered by the mid-sixties. However, studies in monkeys showed that primates are less susceptible; confounding aflatoxin and hepatitis B virus as the aetiology for primary liver cancer in humans led to a scientific debate; thus it lasted to the early nineties before aflatoxins were universally accepted as being a primary cause for liver cancer in humans.

Why has it been so difficult to distinguish mycotoxins and link them to the effects they cause?

Vitamin comparison

Bacteria were first discovered in the period 1660-1670 by Van Leeuwenhoek; he could see them in his experimental microscopes and informed the Royal Society in London about their existence.

Nevertheless, their aetiologic roles in diseases were revealed only some 200 years later; both Louis Pasteur and Robert Koch may take credit for elucidating the link between bacteria and diseases.

A comparison with vitamins is also interesting. Already in 1747, naval surgeon James Lind elucidated the link between citrus fruits and scurvy. Nevertheless, vitamin C was only determined as being the etiologic agent for scurvy in 1912.

In 1905, it was found that Beriberi was prevented by eating unpolished rice, while vitamin B1 or thiamine was already discovered as the aetiologic agent in 1912. By the mid-thirties, most vitamins were known.

What is the common line between bacteria, vitamins and mycotoxins? Firstly, their history shows that scientists should open their mind to investigate the cause-effect-relationship before they can prove the existence of such relationships; the experience with both bacteria and scurvy highlights this issue. The Koch postulates were ground-breaking in this regard.

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Fig. 2. Effect of graded levels of AFB1 on broiler performance (Manegar et al, 2010).

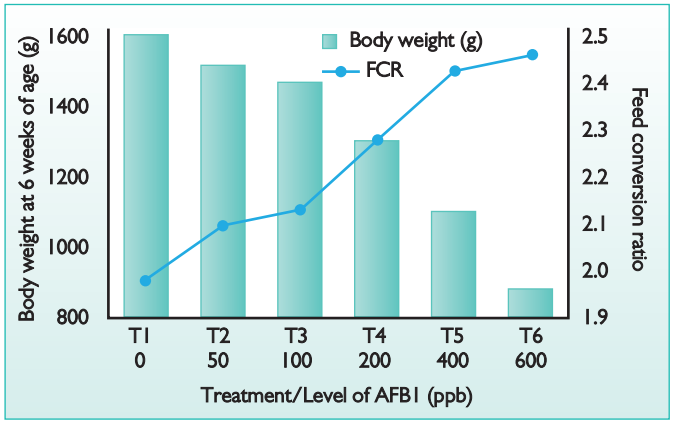
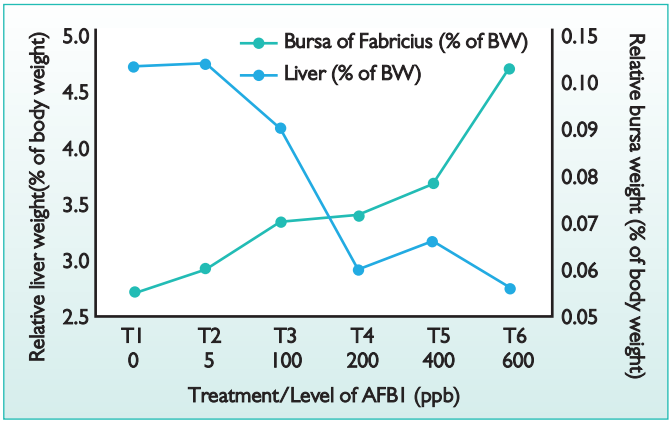


Fig. 3. Effect of graded levels of AFB1 on relative organ weights (Manegar et al, 2010).



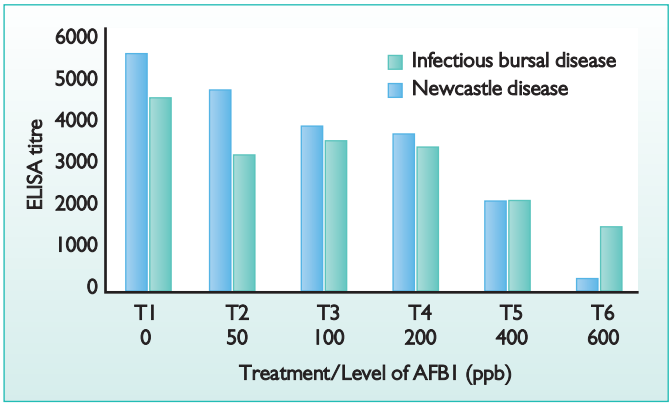


Fig. 4. Effect of graded levels of AFB1 on titres after vaccination (Manegar et al, 2010).

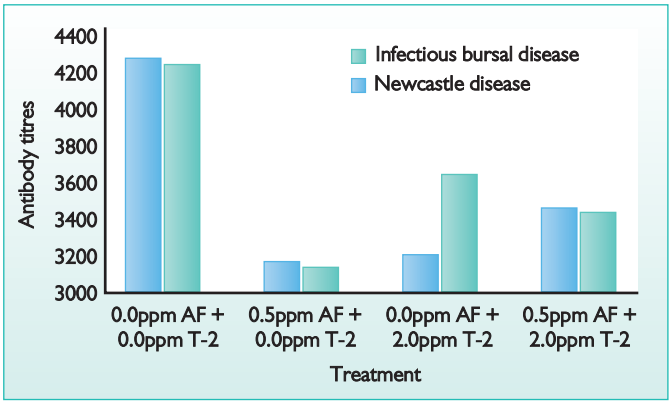


Fig. 5. Effect of aflatoxin and T-2 toxin on broiler vaccinations (Manafi, 2011).

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Secondly, analytical technology must allow scientists to investigate the concerned issues.

It is no coincidence that most vitamins were discovered during the first half of the twentieth century; this is simply the result of assay technology becoming sufficiently sophisticated to detect the ppm quantities (= grams per ton of feed) which are typical for vitamins.

Assay challenge

Today assay technology is still one of the major difficulties scientists face when dealing with mycotoxins; most mycotoxins only occur in feedstuffs in ppb quantities (= milligrams per ton of feed), thus challenging laboratories to work at the edge of their know how.

During the 1960 outbreak of Turkey X disease, the levels of aflatoxin were in the range of 6-15 ppm, which was a feasible goal for the contemporary laboratories, thus allowing elucidation of the cause; an AFB1 level of only 0.5ppm or 500ppb would have remained undetected, since in those days that was still below the detection limit. So, there is a time for everything.

During the early nineties, assay technology was already more sophisticated: Fig. 1 distinguishes the effect of 0.3ppm or 300ppb versus 1.0ppm or 1000ppb of aflatoxin on the mean T-lymphocyte count in peripheral blood; such studies already showed the negative impact of mycotoxins on immunity in a dose-dependent manner.

Effect on vaccination

Later on, further fine-tuning of the assays allowed a further differentiation of the dose-effect relationship, but also allowed distinguishing what happens at fairly low dose rates: it became evident that the immunology is often impaired at far lower dose rates than those producing clinical or performance effects.

Such observations are of particular importance for vaccinations: certain mycotoxins are capable of jeopardising the effect of vaccinations without showing their presence in any other way.

Gliotoxin, for example, is produced by A. fumigatus and C. albicans; this mycotoxin is not known for impairment of production parameters, although it is perceived as immunotoxic; gliotoxin is even used as an immunosuppressive agent after transplantation.

Interestingly, gliotoxin also inhibits the mucociliary system of the lung, which is an aspect of the innate immune system important for removal of airborne particles (such as A. fumigatus spores).

Figs. 2-4 clearly show the effect of graded levels of aflatoxin on performance of the broilers as well as on the development of certain organs (hepatotoxicity + immunosuppression in the bursa Fabricii by AFB1).

Dose related impact

Fig. 4 shows the effect on two of the most common vaccinations; chicks were vaccinated on day five against ND and on day 14 and 24 against IBD, while the ELISA titres were determined at day 42. The negative impact of AFB1 on vaccination efficiency is obvious, in a dose related manner.

This trial also raises another issue of concern – interaction. Even at zero AFB1 contamination, the level of performance is already suboptimal; the causes for this are not highlighted in the concerned paper.

Nevertheless, the paper highlights a topic for discussion: the AFB1 was produced with a pure culture of A. parasiticus in rice; after quantification of AFB1, the rice was mixed in the appropriate concentration in the final feed.

Risk of interaction

Other scientists blame such procedures for their lack of purity; one cannot be sure there is no interaction from other mycotoxins. One can only minimise this risk on interaction by implementation of synthetically purified mycotoxins, which are very expensive.

In the majority of feedstuffs, several mycotoxins co-occur. Therefore, the addition or even synergy of their effects is of practical importance. This is shown in Fig. 5 where the effect of both aflatoxin and T-2 toxin on broiler vaccination is highlighted. The birds were vaccinated against ND on day seven and against IBD on day 14; during the fifth week of age, blood antibody titres were determined by the ELISA technique.

Obviously, both aflatoxin and T-2 toxin impair the effect of vaccination. Such addition or even synergy of effects is a matter of concern. In the meantime, several hundreds of mycotoxins have been detected.

Even though detection limits for individual mycotoxins may be sufficiently accurate to be workable in practice, simultaneously analysing several mycotoxins still remains a problem.

Laboratory scientists are working on procedures to assay some 10 or 11 common mycotoxins in one single procedure, but such techniques remain very expensive.

As long as the focus remains on one single or a few mycotoxins only, underestimation of additional or synergistic effects will remain problematic. In practice, often several mycotoxins are present in feedstuffs even though we lack the analytical means to determine their levels.

Synergistic effect

A more distinguished synergy is shown in Fig. 6. Both CPA and T-2 as well as their combination caused a significant reduction in vaccination response (HI or 'haemagglutination inhibition' titres for ND were reduced by 2.5 to 3.8 logarithmic units).

Fig. 6 also represents a more advanced field of immunology: distinguishing different T-lymphocytes.

CD4+ lymphocytes are also known as 'T helper cells' since they assist or activate other white blood cells (such as macrophages and plasma cells). CD8+ cells are also known as 'cytotoxic T cells' since they directly destroy virally infected body cells or tumour cells.

In this case, the synergistic effect of the two mycotoxins is clearly evident in the thymus; however, in the spleen the effect of the combination was less pronounced than the effect of only administering CPA.

Mycotoxins hamper vaccination – there is no doubt about that. Interestingly, this issue is at the crossroads of two fast progressing domains of science: mycotoxicology and immunology.

The funding of research to protect

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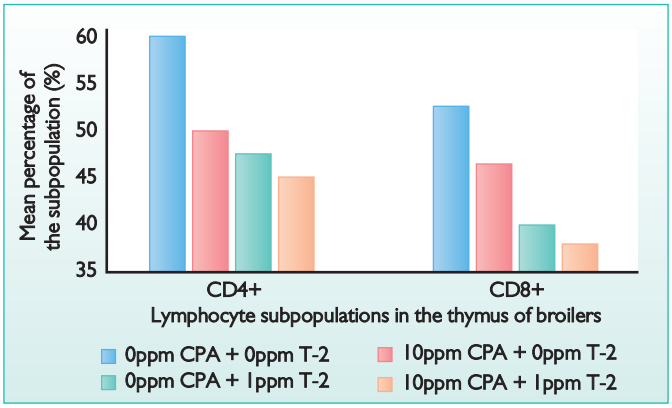
Fig. 6. Synergistic effect of mycotoxins on broiler lymphocytes (Kamalavenkatesh et al, 2005).



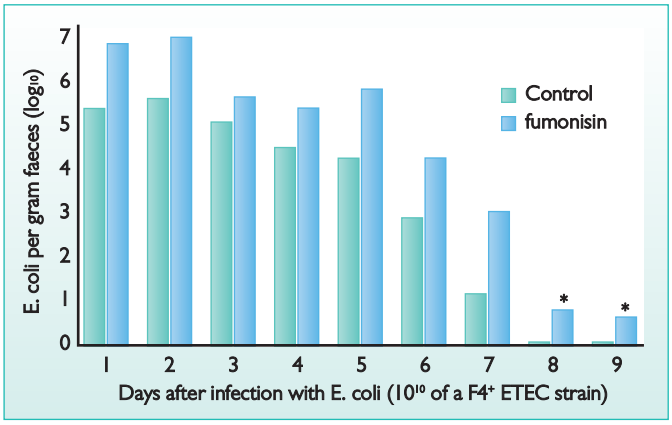


Fig. 7. Effect of fumonisin on excretion of E. coli in piglets (Devriendt et al, 2009).

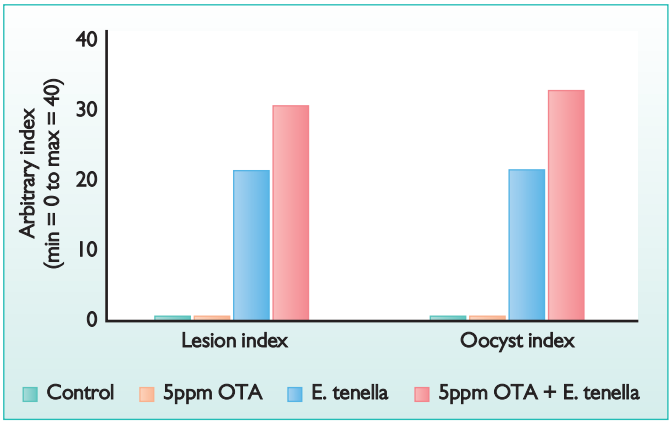


Fig. 8. The effect of ochratoxin on coccidiosis in broilers (Stoev et al, 2002).

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mankind against HIV has induced a huge progress in immunology.

But even though funding is less of a problem in HIV research than it is in animal nutrition, progress is still ongoing: the link between aflatoxins and the breakthrough of AIDS in HIV infected patients was already known during many years; recent research investigated the link between maize and AIDS, thus discovering that fumonisin might be more important than aflatoxin.

A few decades ago, we only could visualise differences between white blood cells by colouring them; nowadays we distinguish huge functional differences within subpopulations of white blood cells, such as the lymphocytes. Such progress creates opportunities, but also contrasting information.

Effects in pathogens

In pigs, apart from reducing performance, both 15 and 83ppb of T-2 toxin clearly hampers several aspects of immunology as reflected by, for example, reduction of macrophages and the integrity of the epithelial lining of the gut.

Both conditions open entrance gates for salmonella. However, it was intriguing that an inoculation with S. typhimurium did not cause a more pronounced disease in T-2 contaminated animals than in the control group. But sometimes, the good comes with the bad: apparently T-2 is also toxic for Salmonella, thus reducing both its multiplication as well as its invasiveness.

Such positive effects of mycotoxins are rare. In most cases, the effect of mycotoxins on the microflora gives an increased pathogenicity.

Effect of fumonisins

As an example Fig. 7 shows the effect of fumonisin on the excretion of EnteroToxigenic Escherichia coli (ETEC) in recently weaned piglets. Although no signs of clinical Fumonisin intoxication were present; the Fumonisin fed piglets showed a 1.1 log unit higher and significantly prolonged excretion of E. coli in the faeces.

Oral immunisation with the concerned E. coli strain was less effective in the Fumonisin contaminated group due to a.o. a reduction in 'antigen presenting cells' (APC) in the gut lining. In other words, fumonisin reduces the efficiency of vaccination in E. coli related weaning problems in piglets.

In broilers, studies show interactions between mycotoxins and common diseases. Fig. 8 shows a relationship between the presence of ochratoxin and coccidiosis: OTA clearly has a bad impact upon both lesions scores and oocyst production after inoculation with E. tenella. The impact of T-2 toxin on the efficacy of anticoccidials was demonstrated a few decades ago.

Hidden aspects

Fig. 9 shows that the impact of mycotoxins on vaccination can occur in a very 'hidden' manner. In this study, AFB1 was administered directly in eggs from broiler breeders. The titres in the one day old chicks represent maternal antibodies.

The chicks were vaccinated on the day of hatching against ND, on day five and 20 against ND and on day 10 and 15 against IBD. Blood sampling was performed on day 28 for determination of HI titres (haemagglutination inhibition is a standard assay procedure in immunology) against both diseases.

The impact of in-ovo administration of AFB1 is obviously detrimental for both maternal antibodies and vaccination efficiency in a AFB1 dose-related manner.

Other trials with mycotoxins in the feed of breeders show similar vaccination problems in their progeny, thus confirming the impact of naturally occurring mycotoxins.

Obviously, there is a carry-over of mycotoxins from the feed to the broiler breeder and subsequently into the eggs. Such carry-over was shown to be detrimental to the vaccination of the broilers.

Thus, mycotoxins can be very nasty. Even though one may perform all possible precautions for optimal performance and efficient vaccination, efforts may not be fully rewarded due to not being able to influence the supplier of day old chicks.

Conclusion

The negative impact of mycotoxins on vaccination efficacy is very obvious. The amount of money spent on vaccination also justifies spending some money on eliminating mycotoxins; this will indirectly improve the efficacy of vaccination as well as directly improving production performance.

Not only aflatoxin hampers vaccination. The majority of the currently known mycotoxins exert a negative impact on immunity. The more research progresses, the more mycotoxins are revealed with an impact on immunity, while the more mechanisms of interaction are revealed.

Such considerations highlight that a simple mycotoxin binder is not the most appropriate choice; although clay minerals may effectively reduce the impact of aflatoxin, there are combination products available that eliminate a much larger range of mycotoxins. Surely, your investment in vaccination deserves the best protection against mycotoxins; therefore, a combination of clay minerals, biopolymers and enzymes is suggested. ■

Fig. 9. Effect of in-ovo contamination with AFB1 on vaccination results in broiler progeny (Sur et al, 2011).