Role of Mycotoxins for Health and the Efficiency of Vaccination in Swine

Mycotoxins are fungal secondary metabolites detected in many agricultural commodities, especially cereals. Pigs are exposed to these toxins due to their high consumption of cereals. In the European Union, regulations and/or recommendations exist in pig feed for aflatoxins, ochratoxin A, fumonisins, zearalenone, and the trichothecenes; deoxynivalenol (DON) and T/HT-2 toxin. The mycotoxins have different toxic effects, but they all target the immune system. They have immune-stimulatory or immunosuppressive effects depending on the toxin, the concentration and the parameter investigated. Ingestion of mycotoxin-contaminated feed can result in an increased susceptibility to infectious diseases, a reactivation of chronic infection and a decreased vaccine efficacy.

High Mycotoxin Levels in Swine: Clear Symptoms, Effect on Performance

Generally, pigs are more sensitive to mycotoxins than any other farm animal. Mycotoxins may cause various toxic effects or mycotoxicosis. Among the trichothecenes, DON and T/HT-2 toxins are the most relevant for the swine industry. The most frequently co-occurring symptoms of high trichotecenes contamination in fattening pigs and piglets are decreased feed intake or feed refusal and diarrhoea. Both feed refusal and diarrhoea might contribute to decreased daily weight gains and worst feed conversion ratio (FCR) in growing pigs.

At high levels, zearalenone is the mycotoxin that is most detrimental to the breeding stock. Young gilts and piglets are the most sensitive group. Fertility problems in swine appear at dietary levels of zearalenone from 100 to 200 ppb. Exposure to zearalenone results in the reddening and swelling of the vulva, increased size of mammary tissue, straining with subsequent rectal and vaginal prolapses, as well as pseudo-pregnancy and false heat. The offspring of affected sows may experience depressed foetus growth in utero, early embryonic mortality and piglets are born with splayed legs. In boars it causes the swelling of the prepuce.

Like other mycotoxins, fumonisin B1 at very high concentrations can cause a variety of species-specific acute toxicological effects in domestic and laboratory animals. It induces pulmonary oedema in pigs and causes hepatotoxicity in all species (Bolger *et al.*, 2001; Haschek *et al.*, 2001). However, in many cases, fumonisin-related symptoms are not easily linked to the effect of fumonisin B1 itself but diagnosed as a secondary problem, such as performance reduction or organ disease due to a different primary reason.

Low Mycotoxin Levels in Swine: Absence of Typical Symptoms, Effect on Health

The clinical toxicological syndromes caused by ingesting moderate to high amounts of mycotoxins have been well characterised. Although ingestion of low to moderate amounts of mycotoxins is common, it generally does not result in obvious intoxication with clear symptoms. It is, however, important to know that these low amounts can result in higher economic losses due to their effect on animals' health. Mycotoxins may impair intestinal health and immune function, and/or cause pathogen outbreak, resulting in altered host-pathogen interactions and thus a different outcome of infection. Indeed, it has been long recognised by veterinary clinicians that marked immunosuppression is observed in livestock ingesting mycotoxins at levels below those that cause overt toxicity (Richard *et al.*, 1978). The immune system is primarily responsible for defence against invading organisms and the effects of low mycotoxin levels are significant to the pigs' health. A suppressed immune function caused by mycotoxins may eventually decrease resistance to infectious diseases, reactivate chronic infection or reduce vaccine and therapeutic efficacy.

The broad immunosuppressive effect of mycotoxins on cellular and humoral immune responses has been demonstrated to decrease host resistance to infectious diseases. In pigs, consumption of feed contaminated with aflatoxin increased the severity of the *Erysipelothrix rhusiopathiae* infection, as demonstrated by the analysis of macroscopic lesions performed after an experimental challenge (Cysewski *et al.*, 1978). More recently, Stoev and collaborators (2000) demonstrated that ingestion of ochratoxin A contaminated feed increases susceptibility to natural infectious diseases in pigs. In this experiment, salmonellosis arose spontaneously in all piglets receiving a diet contaminated with mycotoxin. Low levels of DON have been shown to increase susceptibility to viral infections (Savard *et al.*, 2014). The mucosal immunity, which consists of an innate and adaptive immune system, can be affected by DON (Oswald, 2006). Several studies indicated that DON is able to increase the permeability of the porcine intestinal epithelial layer (Pinton *et al.*, 2009) and affect viability and proliferation of porcine intestinal epithelial cells (Yunus *et al.*, 2012). *Fusarium* mycotoxins, including DON, negatively affect the intestinal reovirus clearance. Li *et al.* (2006) showed that DON and T-2 toxin suppressed the host's immune response to reovirus, as evidenced by the inability to clear the virus from the intestine as well as by increased faecal shedding of the virus. Trichothecenes exposure increased the intestinal viral load, which could increase inflammation and discomfort to the host during the infection process. The increased faecal shedding could enhance virus dissemination among individuals (Li *et al.*, 2006). These results could assume an impact of mycotoxins on host susceptibility to more virulent and aggressive viruses as porcine reproductive and respiratory syndrome (PRRS), PCV2 or PED. It was found that another *Fusarium* mycotoxin, fumonisin B1 was a predisposing factor to infectious disease (Fournout *et al.*, 2000; Oswald *et al.*, 2001). Weaned piglets which received fumonisin B1 had significantly increased colonisation of the small and the large

intestine by the *E. coli*. Exposure of piglets to fumonisin B1 increased the risk for PRRSV disease (Bane *et al*., 1992). More severe histopathological lesions were observed when pigs were exposed to fumonisin B1 and subsequently inoculated with PRRSV. The authors suggest that fumonisin B1 causes immunosuppression, facilitating PRRSV to induce more severe lesions (Ramos *et al*., 2010). Ingestion of low doses of fumonisin B1 revealed pathological alterations of the lungs and an increase of intestinal colonisation by opportunistic pathogenic bacteria in piglets (Halloy *et al*., 2005; Oswald *et al*., 2003).

The effect of mycotoxin intoxication on the reactivation of chronic infection was also investigated. In the immunocompetent host, *Toxoplasma* gondii infection progresses to a chronic phase characterised by the presence of encysted parasites, mainly within the central nervous system or skeletal muscle. Cyst rupture may occur, but the infection remains latent and reactivation is prevented. In immunosuppressed animals, rupture is associated with the formation of new cysts and disease (Suzuki and Remington, 1993). Venturini *et al*. (1996) demonstrated that low and repeated doses of either aflatoxin B1 or T-2 toxin are able to accelerate Toxoplasma cyst rupture in previously infected animals.

An important role of mycotoxins should be highlighted in the aetiology of important diseases such as porcine ear necrosis syndrome (PENS). PENS is usually the result of mixed infections causing damage to the skin. *Staphylococcus hyicus* is the most common isolated agent in lesions of PENS cases, but other pathogens such as *Mycoplasma suis, Streptococcus suis* and spirochetes are often implicated (Thacker, 2006).

Low Mycotoxin Levels in Swine: Influence on the Efficiency of Vaccination

Suppressed immune function by mycotoxins can eventually decrease resistance to infectious diseases, reactivate chronic infections and/or decrease vaccines efficacy (Oswald *et al*., 2005). Therefore, the presence of mycotoxins in swine rations could lead to a breakdown in vaccinal immunity and to the occurrence of diseases such as pleuropneumonia (APP) (Somvanshi and Mohanty, 1991) or adenovirus (Shivachandra *et al*., 2003) or other diseases.

But how do mycotoxins influence the antibody production after the animal is vaccinated? First of all, it is important to know how the pig's immune system works. As is the case in all mammals, the pig's immune system is the major defensive system which should be able to counteract any agents trying to disrupt the organism's integrity. It involves:

1. Innate and non-specific resistance, including both humoral and cell-mediated immunity components. This is the first and quicker barrier for potentially harmful external agents that are trying to damage the body. This involves:
- Surface barriers: mechanical, chemical, and biological barriers;
- Inflammation: eicosanoids and cytokines, which are released by injured or infected cells;
- Complement system: a biochemical cascade containing over 20 different proteins that attacks the surfaces of foreign cells;
- Cellular barriers: leukocytes (white blood cells) that include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils and natural killer cells;
- Natural killer cells: a component of the innate immune system which does not directly attack invading microbes. Rather, natural killer cells destroy compromised host cells, such as tumour cells or virus-infected cells.

2. Acquired specific immunity, which also includes both humoral and cell-mediated immunity components. Acquired immunity develops as the animal is exposed to pathogens (antigen), either actively (by direct stimulation of the immune system) or passively (by transfer of antibodies from milk, colostrum or antiserum, without a stimulation of the immune system). This involves:
- Lymphocytes: cells of the adaptive immune system are special types of leukocytes. B cells and T cells (also called Th cells) are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow. B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune response.
- Immunological memory: When B cells and T cells are activated and begin to replicate, some of their offspring become long-lived memory cells. Throughout the lifetime of an animal, these memory cells remember each specific pathogen encountered and can mount a strong response if the pathogen is detected again. Immunological memory can be in the form of either passive short-term memory or active long-term memory.

Mycotoxins negatively alter both the innate and acquired immunity of pigs, by reducing the phagocytic activity of both macrophages and neutrophils and the humoral and cell-mediated response to antigens. Mycotoxins increase the synthesis of IFNg, a Th1 cytokine involved in the cell-mediated immune response and decreases synthesis of IL-4, a Th2 cytokine involved in humoral response. The alteration of both lymphocyte proliferation and cytokine production might explain the failure in vaccination that is observed *in vivo* (Taranu *et al*., 2005; Figure 1).

The effect of low levels of mycotoxins on the effect of vaccination in pigs was studied by several researchers. It was shown that low dietary levels of mycotoxins reduce the level of antibodies following infection or vaccination, and reduce the activity of phagocytic cells. For example, aflatoxin B1 was shown to interfere with the development of acquired immunity in pigs following erysipelas vaccination (Cysewski *et al*., 1978). In that experiment, pigs fed either a normal diet or an aflatoxin-contaminated diet were vaccinated with erysipelas bacterin and challenged 21 days later with a virulent strain of *Erysipelothrix rhusiopathiae*. In the group of pigs that received the normal diet, three animals were considered

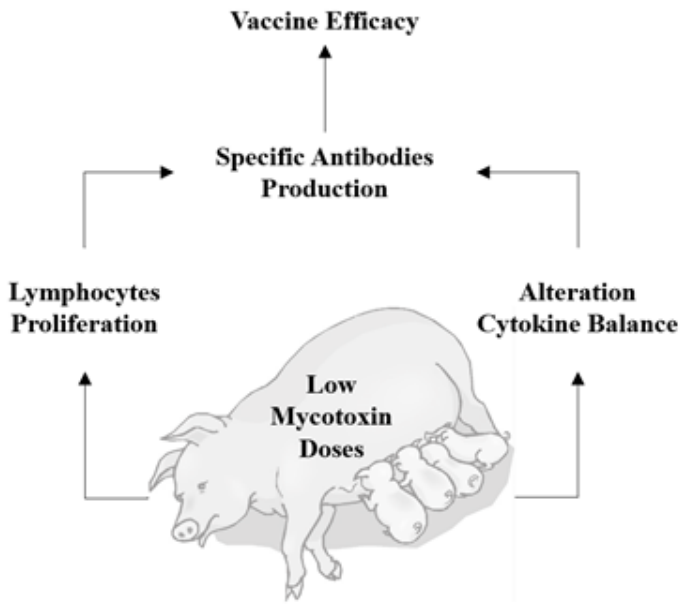

Figure 1. Possible mechanism for the impaired vaccine efficacy in pigs receiving low doses of mycotoxins

immune and two were partially immune following vaccination and challenge. In contrast, in the group of pigs that received the aflatoxin, none of the animals were fully immunised and only one was partially immunised, indicating that aflatoxin consumption interfered with the development of acquired immunity. Taranu *et al*. (2003) also demonstrated that ingestion of low doses of fumonisin B1 decreased the specific antibody response mounted during vaccination. Indeed, a prolonged exposure (28 days) to feed contaminated with 8 ppm fumonisin B1 did not modify the serum concentration of the three immunoglobulin subsets (IgG, IgA and IgM) but significantly decreased specific antibody response to a model antigen. Recently, DON naturally contaminated feed has been shown to decrease porcine reproductive and PRRSV specific antibody responses following experimental infection (Savard *et al*., 2015). Results of this study showed that dietary DON can alter PRRSV vaccine efficacy.

The presence of low levels of mycotoxins in the feed can therefore lead to a breakdown in vaccine-derived immunity and may lead to disease even in properly vaccinated flocks. These reactions are of considerable importance in animal production as effective vaccination programs are relied upon for disease prevention. Practical negative consequences for the pig farm of mycotoxins causing vaccination programmes' failure are:

- The money invested by the farmer in the vaccination programme will be considered as lost, as the disease will have occurred. Vaccine costs correspond to approximately two-thirds of all medicine costs
- The costs due to the occurrence of the disease will be reflected in many factors, representing an enormous burden for the farmer.

As disease occurrence negatively impacts on many factors, calculating its costs in pig farms is almost as complex as explaining the function of the immune system in a simple way. Avoiding disease on pig farms is almost impossible, but prevention is possible, and should be paramount in daily management protocols. Mycotoxins usually found in feeds depress the immune function of the animals, making them susceptible to the pathogenic agents. Preventing negative substances such as mycotoxins will undoubtedly be more cost-effective than treating the diseases triggered by such agents.

Conclusion

In modern animal production, the animal's performance boundaries are continuously being challenged in order to provide the farmer with the highest profitability. Disease outbreaks on farms are known to have economically devastating effects as the whole herd can be affected. These disease outbreaks on pig farms have a direct negative impact on the economic viability, as they represent major cost increases and loss of profit. Disease control and treatment are therefore crucial for the economic viability of pig farms. As described earlier, mycotoxins, the secondary toxic metabolites produced by fungi, negatively impact the swine performance and fertility. Their hazardous effects are even broader, involving the health of contaminated animals as well. It is also clear that at low dietary levels of mycotoxins, the animal's health may be altered and the overall farm profitability reduced due to the increasing susceptibility of animals to infectious diseases and by the decreasing vaccine efficacy. The investigations described in this review clearly indicate that several mycotoxins alter immune-mediated activities in pigs.

Considering the levels of mycotoxins that may be present in animal feeds and human food preparations, further studies are needed to determine the minimal concentration that modulates the immune response. For the time being, considering that mycotoxin dietary levels in Table 1 can lead to the negative consequences related to the animal health, particularly to vaccination failure, is suggested.

Table 1 – Mycotoxin levels in naturally contaminated feed that can lead to the negative consequences related to the pig health, ppb

	AfB1	DON	ZEN	FB1	T2+HT2	OTA
Piglet	>10	>150	>50	>750	>20	>50
Grower/finisher	>10	>250	>100	>1000	>50	>80
Sow, boar	>10	>200	>50	>750	>20	>50

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Radka Borutova studied at the Veterinary University in Slovakia. She obtained her PhD at the same University and the title of her PhD thesis was "Effects of fusarium mycotoxins on antioxidant and immune status in poultry". After her studies she worked at the Ministry of Agriculture: Section of Agriculture and Rural development as Chief state counsellor. In 2009 she joined Biomin Holding GmbH. From May 2013 she works in NutriAd International as Business Development manager for mycotoxin management.

Olga Averkieva PhD, Business Development Manager Mycotoxins, NutriAd International, Belgium. Olga Averkieva was born in Russia and has a PhD in animal nutrition. Among some other jobs she has been technical sales manager for Degussa (Evonik) for the sales of amino acids in Russia for six years. Later she joint Kemin and worked other six years first as Technical Service Manager then as a Product Manager Toxin Binders and Antioxidants in EMEA countries. More than 12 years' experience have given her a good commercial background, knowledge of the market, and very good technical skills in the field of mycotoxins. Since 2013 Olga works in Nutriad as a Business Development Manager Mycotoxins.