Protecting the immune system from one of its major challenges – toxins

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An effective immune system is an important determinant of animal health and well being. Its protective capacity relies on its remarkable ability to distinguish between self and non-self.

Commercial animal production is based on balanced feed providing nutrient requirements and optimised environmental conditions. However, it is very difficult to avoid various nutritional or environmental stresses, which are responsible for immunosuppression and increased susceptibility to various diseases consequently decreasing productivity of farm animals. In this respect mycotoxins are one of the most immunosuppressive factors in animal diets.

Contamination of feed commodities by mycotoxins and bacteria is considered to be one of the most important negative factors in animal feed quality. Numerous researches have concluded that their absorption causes a decrease in performance including decreased growth rate and poor feed efficiency, and are promoting factors of many diseases.

Natural or innate immunity includes physical barriers, specific molecules, and destruction of pathogens by phagocytosis and lysis. Natural immunity is non-specific, local and fast, and gives rise to inflammatory response. The purpose of this article is to study the influence of mycotoxins and endotoxins on this inflammatory response.

The inflammatory response

The innate immunity is present in all animals even before any interaction with pathogens. It is composed of physical barriers (separating the external environment from the inside, such as the skin, mucous membranes, digestive and respiratory tracts) and of the inflammatory response.

Inflammation is usually a beneficial process: its goal is to eliminate the pathogenic agent and repair tissue damage.

However, the inflammatory reaction sometimes exceeds its objectives with deleterious effects as a result. This can be due to the aggressiveness of the pathogen, its persistence, abnormal regulation of the inflammatory process, or quantitative/qualitative abnormality of the cells involved in inflammation.

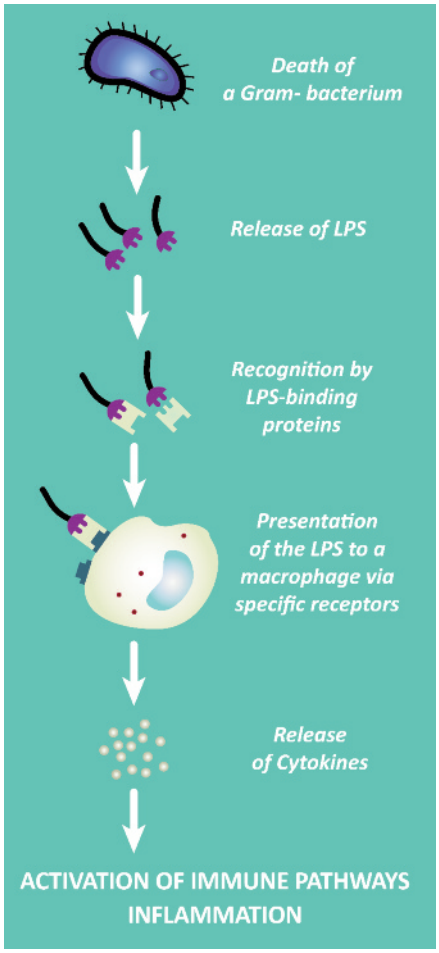

Fig. 1. Role of lipopolysaccharides (LPS) in inflammation.

The causes of inflammation are many and varied: infectious agents, inert foreign substances, endotoxins.

Inflammation begins with a response of 'recognition' involving certain body cells (monocytes, macrophages, lymphocytes) or circulating proteins (antibodies, complement proteins). This recognition phase is followed by the involvement of a whole sequence of cells and mediators which order of intervention is complex and variable.

Some mediators, such as prostaglandins and cytokines are produced by different cell types, acting on several cell types and may control their own production by feedback control. This shows the complexity of the mechanisms of the inflammatory response.

Endotoxins

Endotoxins are lipopolysaccharides (LPS) derived from the cell membranes of Gram negative bacteria and are responsible for its organisation and stability. Although endotoxins are linked within the bacterial cell wall, they are continuously liberated into the environment at cell death and during cell growth and division. Endotoxins are omnipresent (feed, drinking water, air, dust) as a part of bacterial cell wall or as fragments of whole bacteria.

Additionally, the gastrointestinal tract is a large reservoir of both Gram positive and Gram negative bacteria, of which the Gram negative bacteria are a source of endotoxins. Intestinal epithelium is therefore permanently exposed to Gram bacteria, which are able to directly deposit their toxic and pro-inflammatory constituents at the intestinal epithelial apical surface.

These can stimulate localised or systemic inflammation via the activation of pattern recognition receptors.

Endotoxins and inflammation can also regulate intestinal epithelial function by altering its integrity, nutrient transport and utilisation.

Endotoxins do not act directly against cells or organs but through activation of the immune system, especially through monocytes and macrophages, with the release of a range of pro-inflammatory mediators, such as tumor necrosis factor (TNF), interleukin (IL)-6 and IL-1.

These pro-inflammatory cytokines have both local and systemic effects (inflammation, fever and reduction in feed intake).

Moreover, this chain reaction leads to an increase of suppressors of cytokine signalling which have a negative action on growth hormone (GH) induced gene expression in liver.

In short, endotoxins impact the production of IGF1 (insulin like growth factor 1) and alleviate its many actions of growth hor-

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mone (stimulation of cell replication, cell differentiation and the synthesis of cellular products) that have impact on productivity (growth, milk production, fertility).

Mycotoxins

The Food and Agricultural Organization (FAO) estimates that mycotoxins, secondary metabolites produced by fungi, contaminate 25% of the world's agricultural commodities. The presence of mycotoxins alters the quality of agricultural products resulting in economic losses estimated in billions of dollars annually worldwide. The consumption of food and feed contaminated by mycotoxins is a potential health hazard for both humans and animals.

Mycotoxins are one of the most immunosuppressive factors coming from feed, having each of them a different impact on the immune system.

Some of the most toxic or most common types of mycotoxins and their effects are described below:

- **Aflatoxins**: Aflatoxin B1 (AFB1) affects primarily cell-mediated immunity, with a decrease of lymphocytes activity, and phagocytic cell function (mainly macrophages).
- **Ochratoxins:** Ochratoxin A (OTA) mainly acts on humoral immune function, at the level of antibodies synthesis by B-lymphocytes. OTA also interferes with natural killer cells metabolism, resulting in their decreased activity. This immunotoxic effect is significantly dependent upon time of exposure.
- **T-2 toxin** is known to be one of the most toxic trichothecene mycotoxin. It is cytotoxic to lymphocyte cells and results suggest that ingestion of low concentrations of T-2 toxin decreases pattern recognition of pathogens and thus interferes with initiation of inflammatory immune response against bacteria and viruses. It also increases animal to human transmission of pathogens such as salmonella and listeria.
- **Deoxynivalenol (DON)** oral exposure rapidly induces multi-organ expression of pro-inflammatory cytokines, and this is followed by up-regulation of several suppressors of cytokine signalling (SOCS), some of which are capable of impairing GH signalling. DON induces hepatic insulin-like growth factor acid-labile subunit (IGFALS) mRNA upregulation. This effect co-occurs with robust hepatic suppressors of cytokine signalling 3 upregulation. So, oral DON exposure perturbs GH axis by suppressing two clinically relevant growth-related proteins, IGFALS and IGF1. Moreover, DON has been proved to decrease the intestinal expression of claudin proteins and impact the trans-epithelial electrical resistance (TEER), which leads to an increased permeability of the barrier function. This results in an increased risk of trans-epithelial passage of both bacteria and toxins into the systemic system.
- **Fumonisins:** Fumonisin B1 (FB1) impairs both specific (B and T lymphocytes) and non-specific (T lymphocytes, macrophages) immune functions. It causes both stimulation and suppression of responses to foreign antigens, decreases total immunoglobulins, IgG and macrophage phagocyte activity and also decreases the intestinal expression of IL-8. IL-8 is implicated in the recruitment of neutrophils. During inflammatory response, the decreased IL-8 production could lead to an impaired recruitment of neutrophils and so it is associated with an increased susceptibility to enteric infection.

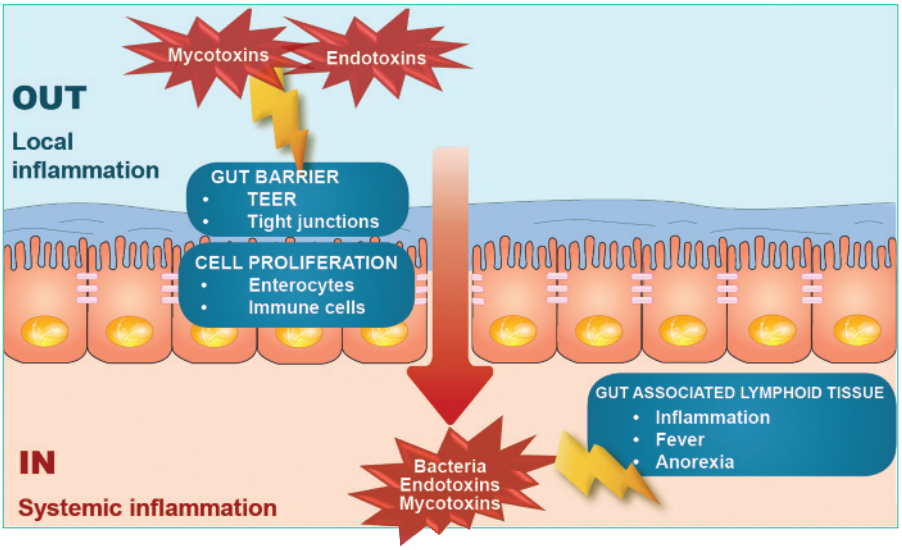

Fig. 2. Combined effects of toxins on gut barrier and inflammation.

Synergies

The chance for multiple mycotoxin contamination of feedstuffs is relatively high and can occur for different reasons. When a synergistic response is present, a rapid and accurate diagnosis of the problem is difficult.

One example of synergy is between DON and FB1. We have seen the effects of DON on the intestinal epithelium, decreasing the villi height and impairing the barrier function of the intestine. Fumonisin reduces epithelial cells proliferation at intestinal level, so the damage provoked by DON is not repaired as there is less cell proliferation, making that low contamination level of DON in presence of fumonisin has a big impact on digestive disorders.

As knowledge increases on mycotoxin and endotoxin action mechanisms, we realise that their effect is far more complex and wide than expected. Regardless of if they act via downregulation of the inflammatory immune response, or by setting-up uncontrolled and unnecessary inflammation, it is clear that limiting the entrance of toxins into

the body is vital in achieving good health and performance in livestock production.

The use of a wide spectrum toxin binder is a key factor to alleviate the above described effects.Demonstration of the effectiveness of a potential mycotoxin detoxifying agent in contaminated feed is often primarily conducted in in vitro conditions. Classical in vitro systems used for that purpose are simple but very far from the natural in vivo conditions. Important factors in relation to the digestion and the fate of feed compounds during passage through the gastrointestinal tract are the composition and pH of gastric and intestinal contents, the gastrointestinal transit conditions and the activity of biochemicals (enzymes) and of the intestinal microflora in the gastrointestinal tract.

The activities of those factors through the gastrointestinal tract are dynamic processes, therefore, these processes cannot be simulated in static in vitro models.

The real challenge is to test on dynamic models. To demonstrate in the most reproducible and reliable conditions the efficacy in vitro of a sequestrant/chelator material, the TNO TIM-1 in vitro dynamic gastrointestinal model can be used. The TNO in vitro gastrointestinal model simulates in high degree the successive dynamic processes in the stomach and small intestine (TIM 1) and in the large intestine (TIM 2).

These models are unique tools to study the fate of compounds during passage through the gastrointestinal tract. The studies for testing mycotoxins detoxifying agents are performed in the TIM-1 system, the TNO dynamic, multi-compartmental system of the stomach and small intestine.

This computer-controlled model simulates the successive dynamic conditions in the gastric compartment and in the three successive compartments of the small intestine. In this system the gastrointestinal conditions were simulated digestive conditions of the pig after the intake of a pig feed.

In 2004, Döll et al, showed that zearalenone is not that difficult to bind compared to other fusarium mycotoxins. Thus, the challenge for a toxin binder is to reduce the absorption of mycotoxins like trichothecenes and fumonisins.

Dr Giuseppina Avantaggiato, from CNR Institute of Sciences of Food Production (ISPA) in Italy has run several trials using this system to evaluate the efficacy of several commercial binding agents and substances potentially useful as chelating agents.

One of the trials carried out in 2004 investigated in vitro screening of 14 adsorbent materials.

Commercial products used to detoxify Fusarium mycotoxins were tested in the pH range of 3-8 for deoxynivalenol (DON) and nivalenol (NIV) -binding ability. Only activated carbon was shown to be effective with binding capacities of 84-95% for DON and 60-63% for NIV, calculated from the adsorption isotherms. The study then used the dynamic laboratory model (TIM) to evaluate the small-intestinal absorption of

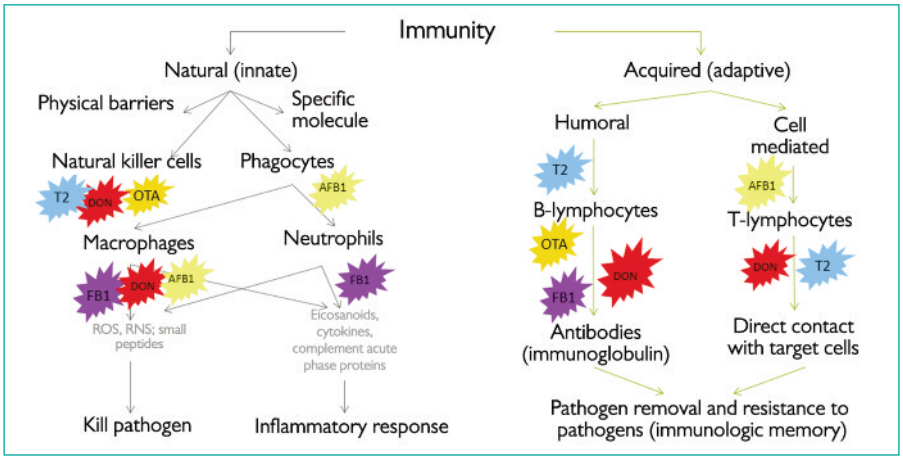

Fig. 3. Impact of mycotoxins on innate and acquired immune systems (adapted from The Mycotoxin blue book, P. F. Surai and J. E. Dvorska p107).

DON and NIV and the efficacy of activated carbon in reducing the relevant absorption.

The in vitro intestinal absorptions of DON and NIV were 51 and 21% respectively. Most absorption occurred in the jejunal compartment for both mycotoxins. The inclusion of activated carbon resulted in a significant reduction in the intestinal mycotoxin absorption. At 2% inclusion level, the absorption with respect to the intake was lowered from 51 to 28% for DON and from 21 to 12% for NIV.

Using information from previous studies it can be concluded that activated carbon appears to be one of the most effective mycotoxin adsorbents. All other commercial products showed poor efficacy in their capacity of binding fusarium mycotoxins. In practice, the use of activated carbon in animal production has some limitations.

High concentrations of activated carbons (>0.5%, w/w) should be avoided in order to minimise the risk of nutrient adsorption as well as an impairment of the caloric/nutritional value of the feed.

Innovative technologies

Specific technologies can modify clay structure at the nano scale, increasing the interlayer space and thus improving its adsorption capacity for larger molecules. This modification can be done by using natural agents such as algal polysaccharides.

Ulvans, polyanionic polysaccharides present in green algae, are sulphated xylorhamnoglucoronans. They are formed by a succession of disaccharides composed of an uronic acid (glucuronic acid or iduronic acid) and sulphated rhamnose. They interact with montmorillonite via silanol groups on the edges of the layers and compensation cations in the interlayer space of montmorillonite. The presence of ulvans in the interlayer space of the montmorillonite increases the accessible adsorptive surface and the number and types of adsorption sites, resulting in a matrix similar to the structure of activated carbon. The adsorption of mycotoxins in this innovative and worldwide patented (Olmix) material is a complex mechanism involving CEC (cation exchange capacity) and surface area of montmorillonite, the polyanionic structure of ulvans and the 'microtubular' structure formed in the interlayer space, allowing ionic and hydrophobic interactions with mycotoxins.

When testing this new material using the TIM-1 system in TNO, results were even better than those obtained with activated carbon for big mycotoxins such as DON and Fumonisin. In addition, the use of this product did not impair the bioaccessibility of nutrients.

Conclusion

It is predicted that there will be 9,000 million inhabitants on Earth in the year 2050. Population feeding is a challenge not only from the quantitative point of view but also from a qualitative point of view. To grant healthy food, free of potential harmful agents and the quantity to feed the population is a challenge that demands changes and efforts from all participants in the food chain.

Globalisation and international trade enable the movement of raw material to the consumption site worldwide. Climate change, storage conditions and livestock industrialisation have increased the concentration and distribution of toxins in feed and food.

To avoid the presence of mycotoxins and endotoxins in the feed supply is becoming almost impossible and this has a deep impact on animal health and performance.

A tool to decrease the chances of these toxins entering the animal organism is more than needed. The use of wide spectrum toxin binders in feed and optimum farm management practice is the only possible method to reduce toxins effects and their impact in the food chain. This will reduce the problems associated with animal performance and human health. ■

References are available from the authors on request