4.2. Contaminants other than mycotoxins

By Margaux Lecolinet

Endotoxins

Endotoxin is the common name given to lipopolysaccharides (LPS), a structural pattern of the outer membrane of Gram-negative bacteria such as *Salmonella, Pseudomonas, Escherichia*. LPS is composed of three parts: lipid A, the core oligosaccharide, and the O antigen. It is an agonist of the TLR4 (Toll-like receptor 4) receptor, present at the surface of leukocytes (monocytes, macrophages, heterophils) which activate the immune response (Bertani and Ruiz, 2018). When TLR4 is triggered, it activates NF-κB which is a DNA transcription factor responsible for triggering the production of pro-inflammatory mediators such as TNF-α, IL-6, IL-1b and nitric oxide (Zhang and Wang, 2014), as shown in Figure 4.2.1. Endotoxins therefore initiate a pro-inflammatory response in the gut.

Endotoxins can be produced by the rumen or gut microbiota. But they can also come from the environment, feed, and milk in particular, which could impact young animals. The effect of LPS will differ greatly depending on the dose and the individual health status. It seems that LPS can be well tolerated in healthy individuals. Contrarily, when the inflammation has begun, at high doses, LPS can enter the bloodstream and induce septic shock. But between these two scenarios, there can be quite a bit of variation. In addition to favoring gut inflammation, LPS can be involved in dysbiosis (Candelli *et al.*, 2021). These variations in symptoms have been partially explained. First, not all LPS are equivalent; different strains of bacteria can produce this molecule, which, in turn, can have slightly different structures. For instance, in humans, it has been shown that *bacteroides* produce less harmful LPS than *E. coli* (Vatanen *et al., 2016*). The origin of LPS could play a role in intestinal immune homeostasis (Steimle *et al.*, 2019). Therefore, the composition of the gut microbiota, responsible for the production of endotoxins, plays a major role in the toxicity of LPS.

The innate immune signaling pathway responsible for the detection of LPS is a fine-tuned evolutionarily conserved mechanism. However, differences in the metabolic pathways do exist (Keestra and Van Putten, 2008). Depending on the species, animals can have slightly varying sensitivities toward LPS. In monogastric species, LPS are mainly a product of gut bacteria. Birds are in general more tolerant to LPS (Berczi *et al.*, 1966) due to a difference in their LPS signaling pathway. In mammals, when TLR4 is activated, it uses two separate metabolic pathways: MyD88 and TRIF, also called dependent or independent My-D88. In birds, the independent-MyD88 pathway is not activated (Keestra and Van Putten, 2008), potentially due to the absence of the TRAM intermediary (Karpala *et al.*, 2008), as shown in figure 4.2.1, which could explain the difference in sensitivity.

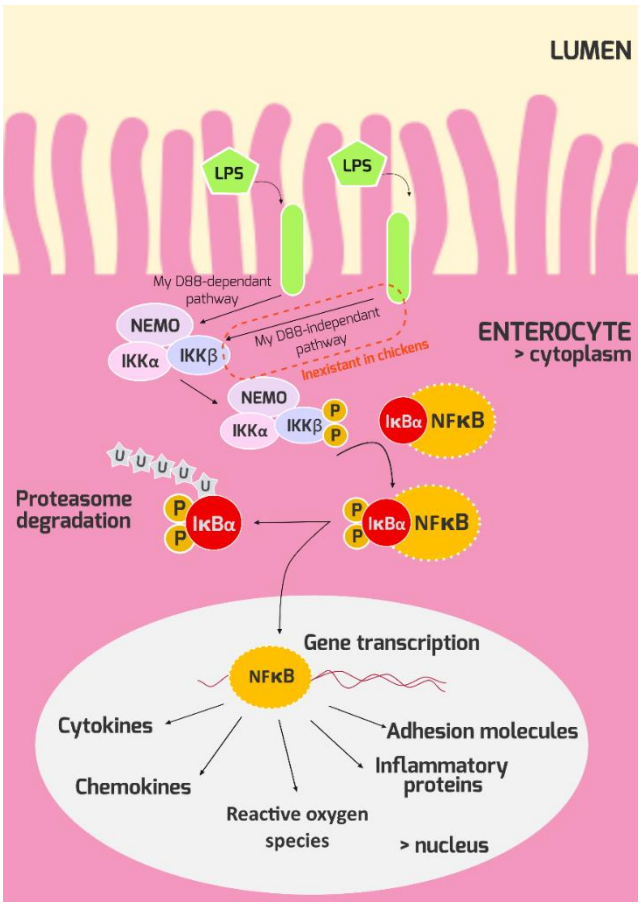

Figure 4.2.1. LPS induce a pro-inflammatory status in the gut (model adapted from Palsson McDermott and O'Neill, 2004)

In ruminants, LPS are mainly a product of gram negative bacteria inhabiting the rumen. Current studies tend to show that ruminal LPS exhibit immunomodulatory properties (Sarmikasoglou and Faciola, 2022). However, it is shown that LPS are linked to acidosis in dairy cows fed a high-concentrate diet. During acidosis, as the pH decreased, cell lysis increases in the rumen which causes a rise in LPS levels (Kent-Dennis *et al*., 2020). Ruminal LPS translocate to the bloodstream and can provoke liver injury and pro-inflammatory status (Guo *et al*., 2017).

Recently, some researchers have tested the ability of certain ingredients to bind endotoxins and reduce their load in the gut. Most studies involve clays using *in vitro* protocols. There is great variation in efficacy; some ingredients have shown promising results, but some are inconclusive in binding LPS. In addition to the heterogeneity found in ingredient efficacy, LPS binding efficacy varies depending on the origin. For instance, the same clay can have a good adsorbing capacity for LPS from *Salmonella* and a very poor one for LPS from *E. coli* (Adisseo internal report, 2022).

LPS binding strategies combined with intestinal support via beneficial bacteria shows promise. Indeed, it has been shown that LPS toxicity depends on the bacterial strain. Avoiding the development of pathogenic bacteria and maintaining the homeostasis of the gut microbiome could reduce or even prevent the production of highly toxic endotoxins in the gut (Candelli *et al*., 2021). The use of probiotics, SCFA's or MCFA's should be further investigated in livestock farming.