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Endotoxins in swine - effects and strategies for control

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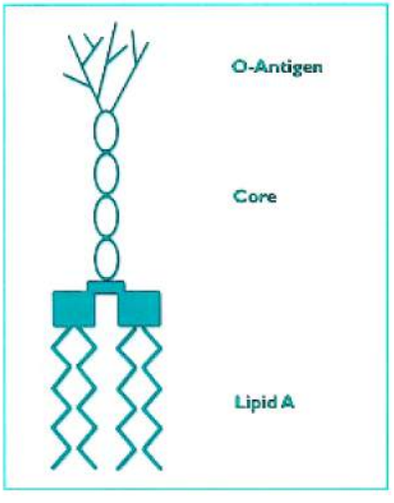
Endotoxins are structural components of bacteria. They are part of the outer membrane of Gram negative bacteria., which are released mainly when bacteria are lysed, due to the use of antibiotics or because of the body's defence mechanism. Together with phospholipids and membrane bound proteins they are constituents pf the outer cell membrane the typical structure of an endotoxin consists of a lypopolysaccharide (LPS). These LPS define many of the properties of host-parasite interactions.

LPS consists of three structural elements. One is a hydrophobic component, called lipid A, which serves to anchor the molecule into the membrane. The second is a core oligosaccharide. The third component is a hydrophilic O-polysaccharide projecting into the extracellular space. More than 150 different variants of the third component are known. The O-polysaccharide portion seems to be relevant to host-parasite interactions because its disappearance results in loss of virulence.

The loss of the proximal part of the core oligosaccharide induces bacteria to become extremely sensitive to detergents, antibiotics and bile salts. So it seems that this region is essential for the maintenance of outer membrane functions as a biological barrier.

Mutations altering the lipid A component are mostly not viable, suggesting that it is important for the maintenance of outer membrane integrity as a whole.

Fig. 1 Lypopolysaccharide structure



Lipid A and virulence

The physiological activities of LPS are mediated mainly by the lipid A component of LPS. Lipid A is a powerful biological response modifier that can stimulate the mammalian immune system. During infectious disease caused by Gram negative bacteria, endotoxins released from or part of multiplying cells have similar effects on animals and significantly contribute to the symptoms and pathology of the disease encountered.

Since lipid A is embedded in the outer membrane of bacterial cells, it probably only exerts its toxic effects when the bacteria are lysed as a result of autolysis and the membrane attack complex (MAC), ingestion and killing by phagocytes, or killing with certain types of antibiotics.

The injection of living or killing Gran negative cells or purified LPS into

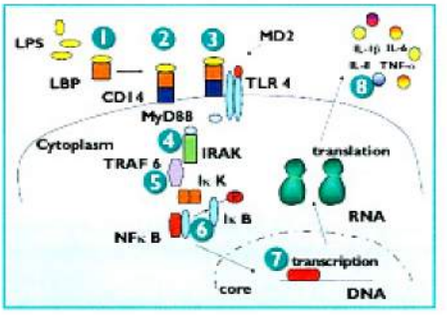


Fig. 2 Schematic view of the cellular endotoxin (LPS) signal transduction pathway . LPS bindind protein (LBP) solved in the plasma ligates at LPS (l). This complex binds to the cell surface receptor CDI4 (2). Aggregation of LBP/LPS/CDI4 complex with the protein MD2 and the transmembrane toll like receptor (TLR) 4 (3) induces the signal transduction cascade in the cell (4,5). Finally, transcription factor NFxB is activated (6) and starts translation of several genes to proteins, for example pro-inflammatory mediators like IL-IB (7,8)

experimental animals causes a wide spectrum of non-specific pathophysiological reactions, such as fever, changes in white blood cell counts, disseminated intravascular coagulation, hypotension, shock and death. Injection of fairly small doses of endotoxin results in death in most mammals.

The sequence of events follows a regular pattern:

- Latent period.
- Physiological distress (diarrhoes, prostration, shock).
- Death.

How soon death occurs varies on the doses of the endotoxin, route of administration, and species of animal. Animals vary in their suscep-

Treatment	Final weight (g)	ADG (g)	Feed intake (g/d)	CI
Control	749.68±14.16[a]	33.61±0.66[a]	44.96±0.88[a]	1.388±0.010[b]
LPS	684.63±16.81[b]	30.51±0.79[b]	42.58±0.97[ab]	1.396±0.018[a]
YCL	744.40±19.12[a]	33.35±0.91[a]	44.28±1.04[a]	1.328±0.012[b]
LPS + YCL	692.93±8.25[b]	30.91±0.38[b]	41.56±0.58[b]	1.344±0.014[b]

Means in the same column with a different letter are statistically different (P<0.05)

Table 1. Effect of inclusion of yeast cellwall in the diet on production parameters (1-21 days) of broilers inoculated with LPS of E. coli (Badia R. st al).

tibility to endotoxin and the mechanism is complex.

In humans, LPS binds to a lipid binding protein (LBP) in the serum which transfers it to CD 14 on the cell membrane, which in tum tein MD2, which associates with toll-like receptor-4 (TLR4).

This triggers the signaling cascade for macrophage/endothelial cells to secrete pro-inflammatory cytokines and nitric oxide that lead to the characteristic "endotoxic shock".

CDI 4 and TLR4 are present on several cells of the immunological system, including macrophages and macrophages, three types of events are triggered during their interaction with LPS.

•Production of cytokines including IL-I, IL-6,IL-B tumour necrosis factor (TNF) and platelet activating factor. These, in turn leukotrienes. These are powerful mediators of inflammation endotoxin toxaemia. LPS activates macrophages to enhanced phagocytosis and cytotoxicity. Macrophages are stimulated to produce and release lysosomal enzymes. IL-1 (endopenous pyropen) and tumour necrosis factor (TNFalpha), as well as other cytokines and mediators.

•Activation of the complement cascade. C3a and C5a cause histamine realese (leading to vasodilation) and affects neutrophil chemotaxis and accumulation. The result is inflammation.

•Activation of the coagulation cascade. Initial activation of Hageman factor (blood-clotting Factor XII) can activate several humoral systems resulting in:

-Coagulation: a blood clotting cascade that leads to coagulation, thrombosis, which deplete platelets and various clotting factors resulting in internal bleeding.

-Activation of the complement alternative pathway (which leads to inflammation)

-Plasmin activation which leads to fibrinolysis and haemorrhaging.

-Kinin activation releases bradykinis and other vasoactive peptides which causes hypotension.

The nest effect is to induce inflammation, intravascular coagulation, haemorrhage and shock. LPS also acts as a B cell mitogen, stimulating the polyclonal differentiation and multiplication of B-cells and the secretion of immunoglobulins, especially IgG and IgM.

Control of endotoxins

In general, strategies to control endotoxin contamination in animals include all of those aimed at the reduction of bacterial contamination. These strategies include, but are not limited to, biosecurity, use of prebiotics, probiotics and improved nutrient digestibility. Other strategies such as vaccination and use of toxin binders specifically target endotoxin contamination.

• **Vaccination.** As explained before, the lipid A portion is responsible for endotoxin toxicity. Also it is the more consistent portion of LPS structure. Currently, immunisation against lipid A is being developed, but the high cost makes it a non-viable option for livestock production. Another option considered in vaccination is to immunise against LBP, in an attempt to reduce the formation of LBS-LBP complex that initiates the cascade of events leading to pathogenesis. This option is also expensive and currently only to be considered for human use.

• **Immunity modulators.** Use of immune modulators to compensate the effects of endotoxins have been tested in animal production. In broilers, inoculation with LPS induces an activation of the immune system. Some studies show that broilers inoculated with LPS decrease productivity. This reduction is related to the action of interleukins produced during the acute inflammatory phase. On the other hand, some studies show the immune-modulating action of B-glucans present in yeast cell wall. Table I shows the results of using yeast cell wall (YCL) in diet of broilers inoculated with LPS. YCL was capable of counteracting the effects of LPS in conversion index (CI). Further tests would be needed to study the possibility of using immune modulators against endo-

Table 2. Endotoxin concentration in blood of sows before and after (Three weeks) addition (lkg/MT of feed) of Mycoad AZ (Biocheck, Leipzig)

Sow number	Endotoxin ml/L Before binder	After binder
123	4.55	<0.05
125	30.86	<0.05
126	<0.05	<0.05
127	>50	<0.05
128	22.88	<0.05
129	2.85	<0.05
130	47.33	<0.05

toxins.

A more practical approach to reduce binders. Toxin binders are widely used to control other toxins such mycotoxins. The binder and the mycotoxin form a complex that is too large to be absorbed into the blood system. The complex is then eliminated in the faeces.

Most mycotoxin binders are hydrophilic molecules (bentonites, aluminosilicates) efficient at capturing polar molecules such as aflatoxin. The capacity of These traditional mycotoxin binders to capture more lipophilic-like molecules such zearalenone or DON is questionable. There are also organic mycotoxin binders (MOS based) that claim to be able to absorb a wider range of mycotoxins.

When considering the possibility of using toxin binders to capture endotoxins, the ideal binders candidate should target lipid A. The reason being that lipid A is responsible for the pathogenic effects of endotoxins, and it is the portion of the structure that remains constant across different endotoxins, so by targeting lipid A the toxin binder would have a wider range of action.

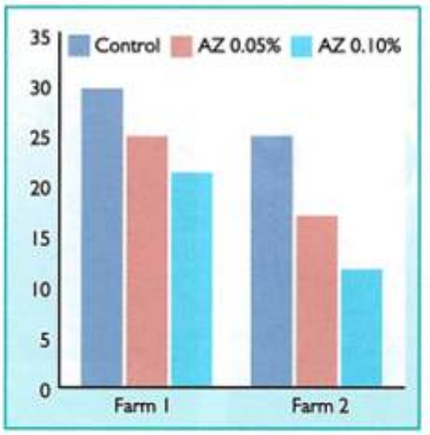


Fig 3. Endotoxin concentration in bacterial cell culture of sow faeces (1-2pp) after in vitro supplementation of different Mycoad AZ concetrations (University of Leipzig).

Some preliminary studies have already tested the posibility of using toxin binders against endotoxins is swine. Fig 3 the results of using a commercial available toxin binder to reduce free endotoxin concetration in endotoxin producing bacterial cell culture.

The product was capable of binding endotoxins at two different inclusion rates. This biding capacity was confirmed in an in vivo test.

Table 2 shows free endotoxin concentration in blood of shows before and after feeding them the commercial toxin binder.

This preliminary data suggest than the toxin binder is capable of biding endotoxin and the gastrointestinal tract, and thus preventing endotoxins from being absorbed.

Further studies are currently underway to further test this product.

Summary

Endotoxins have a wide variety of effects on livestock affecting performance parameters. Different means of control of endotoxins have been tested, but most of them are too expensive to be considered in animal production.

A cost effective method would be the use of toxin binders to capture endotoxins in the gastrointestinal tract and preventing them from entering the blood systems. Some studies are underway to test this possibility.