Newsletter

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> EDITORIAL

Endotoxins are incredibly fascinating substances. On the one hand they stimulate the immune system in a positive way; on the other hand they cause endotoxic shock and death. Although a lot of research has been done in the last years it has not yet been possible to fully understand the exact transition point of "good to bad". One reason could be that endotoxaemia is mostly a subsequent effect of other underlying illnesses in humans and companion animals and that the individual immune constitution plays a fundamental role in this response. Of special interest for pig farmers is the involvement of endotoxins in, for example, the Mastitis- Metritis-Agalactia Complex of sows or the sudden death syndrome in piglets. But these are just two examples of endotoxin-associated diseases which can lead to financial losses and increased labour for farmers. These out of many more examples were the reason why BIOMIN started to investigate in this topic, allowing a better understanding of the harmful action of endotoxins leading to the development of feed additives which help keeping endotoxins under control.

Endotoxins are produced by bacteria. This is of main interest when one imagines that bacteria are part of our lives. They are everywhere around us as well as in our gastrointestinal tract or in other parts of hollow organs. As long as there is a balance between growth and death of bacteria every process remains normal in the organism but what happens when this balance is disturbed? What happens when more bacteria than those the organism can cope with are produced? In this case bacteria have the chance to liberate their poisonous substances or compete against the body's defenses consequently harming it.

MSc. ***Simone Schaumberger***

Mycofix® product line

As the classification of bacterial toxins is very complex a schematic overview of the differences between endotoxin vs. enterotoxin is presented using the example of *E. coli (Table 1). E. coli* are Gram-negative bacteria common in human and animal environments. Most people know them as they cause different kinds of diarrhea and take part in flu or other infectious diseases.

ENDOTOXINS – Silent but harmful: Demystifying misconceptions

Depending on their characteristics bacteria produce endo- or exotoxins (*Figure 1*). Unlike mycotoxins, bacteria proliferate in feed and in humans and animals (GI-tract, wounds, urinary tract, to name a few); therefore representing another important group of

Table 1 *- Main differences between endo- and enterotoxins*

ENDOTOXINS	ENTEROTOXINS
• Protein complexes as part of the cell wall of *Gram-negative* bacteria • Released during death, lyses and bacterial growth • Affect complex immune and inflammation cascades in the organism • Result in fever, influenza like symptoms and in worst case shock and death • Less potent and less specific as they do not act enzymatically	• Form of exotoxin • Proteins excreted by *Gram-negative* and *Gram-positive* bacteria • Excreted by living bacteria • Affect the gastrointestinal tract • Result in diarrhea • Different kind of enterotoxins: heat labile and stable forms

toxins in terms of animal production. To better understand their action, a definition of endotoxins versus exotoxins is given in *Table 1*.

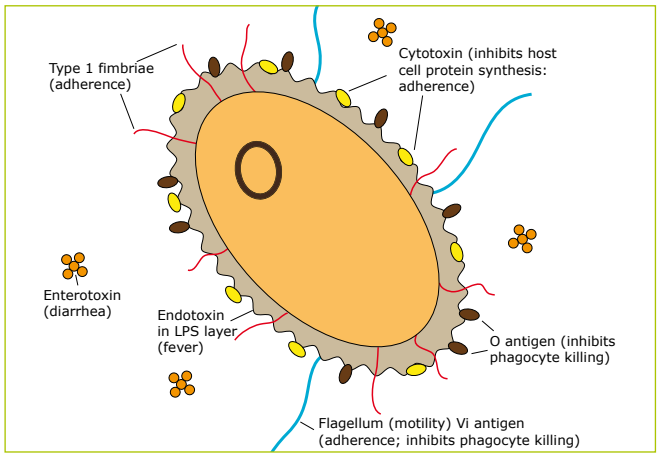


Figure 1 *– Enterotoxins and endotoxins in E. coli*

- ***Exotoxins (Ectotoxins)***: Exotoxins are soluble proteins excreted by a microorganism, including bacteria, fungi, algae, and protozoa. An exotoxin can cause damage to the host by destroying cells or disrupting normal cellular metabolism. Both Gram-negative and Gram-positive bacteria are able to produce exotoxins. They are highly potent and can cause major damage to the host. Exotoxins may be secreted, or, similary to endotoxins, may be released during lysis of the cell. Exotoxins which react with cells of the small intestine and cause diarrhea and gastroenteritis, are called *enterotoxins*. They are proteins excreted by different bacteria. Examples for enterotoxins in *E.coli* are (*Figure 2*):
  - ETEC: enterotoxic *E. coli* (A)
  - EPEC: enteropathogenic *E. coli* (B)
  - EIEC: enteroinvasive *E. coli* (C)
  - EHEC: enterohemorrhagic *E. coli* (D)

All of these toxins are able to induce different kinds of diarrhea depending on their invasion mechanism: ETEC is known as traveler's diarrhea and the hurtful toxin is heat-labile (LT 1 and LT 2). EPEC is an important toxin in piglets or infants diarrhea which leads to growth dysfunction. EIEC are very aggressive and destroy the gut mucosa and so shigellosis condition appears.

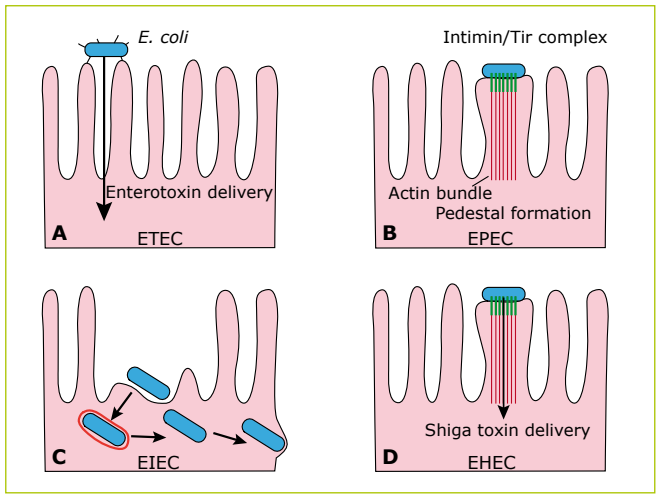


Figure 2 *- Examples for enterotoxins in E.coli*

EHEC induces a dangerous bloody diarrhea.
Other potent exotoxins – named after their place of action - are the neurotoxin of *Clostridia*, which do harm to the central nervous system representing one of the most potent natural toxins. Besides neurotoxins and enterotoxins, there are other forms called hemotoxins (destroy red blood cells) and cardiotoxins (damage heart muscle).

- ***Endotoxins***: Classically, an endotoxin is a toxin that, unlike an exotoxin, *is not* secreted in soluble form by live bacteria, but instead is a structural component in the bacteria which is released mainly when bacteria are lysed. Endotoxins are commonly referred to in literature as lipopolysaccharides (LPS). The toxic and non variable part is the Lipid A (identical in all cell walls of Gram-negative bacteria). Endotoxins, unlike exotoxins, react with different blood proteins, cytokines (involved in the immune response), amongst others, thus inducing immune reactions. The complex cascade endotoxins induce is schematized in *Figure 3*.

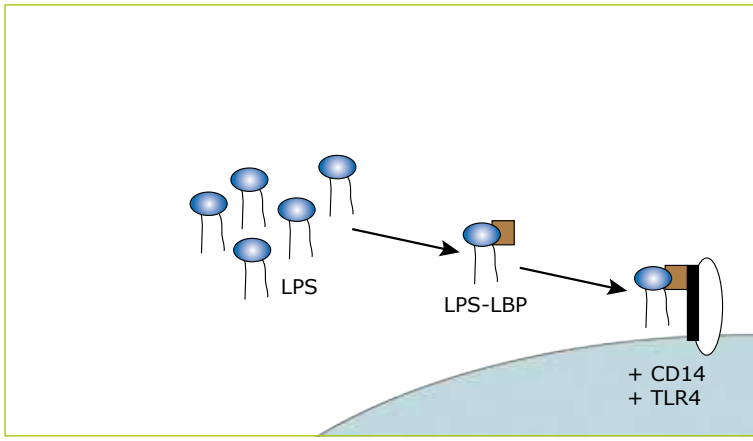


Figure 3 *– Mechanism of host response to endotoxins. Once internalized, this new complex activates TLR4, followed by initiation of the innate (3a) LBP: Lipopolysaccharide-bindig protein, CD14: Cluster of differentiation*

Endotoxins are part of the outer membrane of the cell wall of Gram-negative bacteria (*e.g. E.coli, Salmonella, Shigella, Pseudomonas,* amongst others) independently of the fact if the organisms are pathogenic or not (*Figure 4*). The toxicity is associated with the lipid component (Lipid A) and the immu-

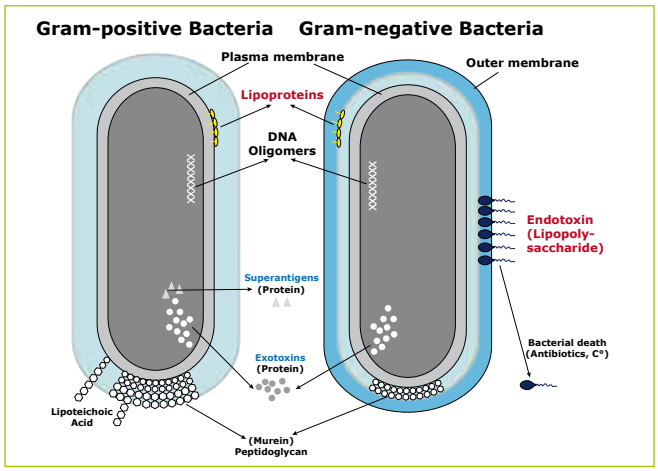


Figure 4 *- Differences between Gram-positive and Gram-negative bacteria*

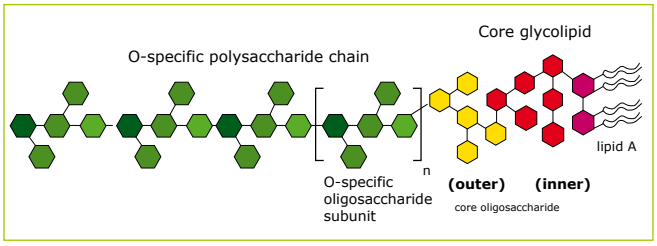
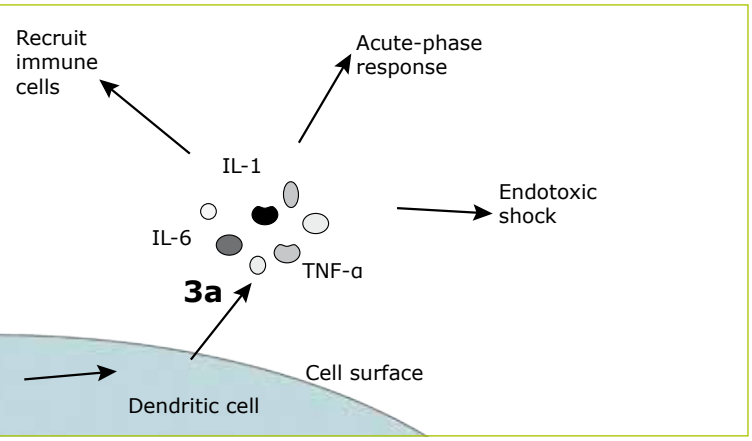


Figure 5 *- Gram-negative bacterial endotoxin (lipopolysaccharide, LPS) structure*

nogenicity is associated with the polysaccharide components (*Figure 5*). LPS elicits a variety of inflammatory responses in animals and activates the complement by the alternative pathway, so it may be part of the pathology of Gram-negative bacterial infections.

Compared to the classic exotoxins of bacteria, endotoxins are less potent and less specific in their action, since they do



endotoxins are bound by LBP and transferred to CD14; immune response. LPS: Lipopolysaccharide (endotoxin), 14, TLR4: Tool-like receptor

not act enzymatically. Since Lipid A is embedded in the outer membrane of bacterial cells, it probably only exerts its toxic effects when released from multiplying cells or when bacteria are lysed as a result of autolysis, ingestion and killing by phagocytes or certain types of antibiotics.

Natural sources of endotoxins are:

- Exogenous:
  - Air, food, water
  - Faeces, urine
- Endogenous:
  - Colonized mucosa
  - Gastrointestinal tract
  - Wounds, traumas
  - Abscesses
  - Bacteria in blood and lymph
  - Fat tissue mobilization

Therefore, except in the case of septic infections, endotoxaemia is no independent disease but a consequence of an underlying illness or a capacity overload.

Released LPS reach the circulation and, in healthy animals, the endotoxin is bound to different serum constituents and lipoproteins and delivered to the liver and neutralized (liver cells), stored (fat tissue) or eliminated (mammary gland, gut, lung).
In the case of, for example, damage in the gut barrier and raised permeability (e.g. constipation – feces stay in the gut longer and so microbes can proliferate), blood circulation has to combat more endotoxins at the same time. As long as the liver (main detoxification organ) and organs are "healthy" the body is able to detoxify LPS. But when the point is reached when the liver cannot cope with all the high endotoxin challenge, many metabolic, immune and endocrine reactions are triggered, leading, in the worst case, to an overshoot reaction and even death.
The immune cascade includes a fever reaction: LPS bound to plasma proteins is recognized by monocytes and in this way cytokines are activated, inducing fever. So in animals, raised body temperature may be the easiest way to detect an endotoxin reaction.

Stimulating factors for endotoxin-associated diseases:

- Stress: heat, environment
- Wrong feed formulation
- Constipation
- Intestinal flora – dysbiosis
- Gut lesions (bacteria, virus,..)
- Lack of hygiene
- Antibiotics

Because of the reasons stated above, the constitution and the immune status of individuals play a very important role in the output of the endotoxin cascade. Furthermore, as most endotoxin charge can be found and is liberated in the gut due to the presence of many bacteria, the importance of a healthy gut, reached by adequate food and good environmental conditions, is crucial.

Prevention against endotoxins:

- Maintenance of the gut function in a good status – right feed formulation
- Use of antibiotics in combination with anti-inflammatory drugs when treating for example MMA or other diseases
- Control of body temperature (fever is an early response mechanism) and examination of feces of sows around birth
- Low dust and dirt concentrations – maintenance of both the stable and animals clean (eg. cleaning animals before moving them between stables).

The Mycofix® product line offers a complex-strategy solution also for the counteraction of endotoxin effects. Adsorption components enable the binding of endotoxins. Furthermore, the biological constituents stimulate the production of the anti-inflammatory cytokine (IL-10) and the macrophage activity and inhibit the production of pro-inflammatory cytokines (IL-6 and TNF-α). Moreover, the algae extracts incorporated in the Mycofix® product line seems to be very effective in inhibiting pro-inflammatory cytokines (IL-6 and TNF-α) production. The plant extracts inhibit pro-inflammatory cytokine (IL-6) production.

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