ENDOTOXINS AND THEIR ADSORPTION USING MINAZEL, MINAZEL PLUS AND MYCORAID

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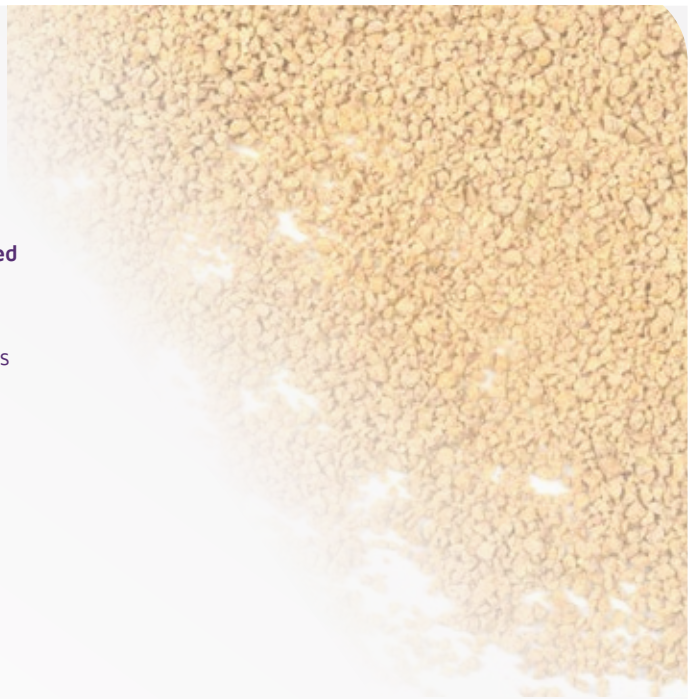


Feed ingredients and animal feed can be contaminated with various chemically and biologically derived residues, including dioxins, heavy metals, microbes, endotoxins, and mycotoxins. Among these contaminants **little is understood about endotoxins**. In this article the following areas on endotoxins are covered:

1. Endotoxin structure and effects on animals
2. Prevention of endotoxin absorption
3. Adsorption of endotoxins using Minazel, Minazel Plus and MycoRaid

Endotoxin structure and effects on animals

Endotoxins are lipopolysaccharides (LPS) found on the outer membrane of Gram-negative bacteria. They are structural components of the bacterial cell wall and they form part of the building blocks of the outer membrane of these bacteria.

- Endotoxins are not inherently toxic per se in the same way that exotoxins. However, they induce **strong inflammatory responses** that can damage the host and may even be lethal.
- Endotoxins are **released into the environment** when the bacteria multiply or when their cell membranes rupture through bacterial lysis.

LPS are chemically composed of an **outer O-polysaccharide chain (O-antigen)**, an **inner R-polysaccharide chain** and **lipid A** (*Figure 1*).

The **O-antigen varies** between different bacterial strains and is the basis of the **serological typing of Gram-negative bacteria** such as *E. coli*.

Lipid A is the most bioactive part of the molecule and it is a more conserved structure. However, there may be structural differences between bacterial strains and species that alter the way host cells respond to them.

Alterations to the Lipid A may allow pathogenic bacteria to evade the host immune response.

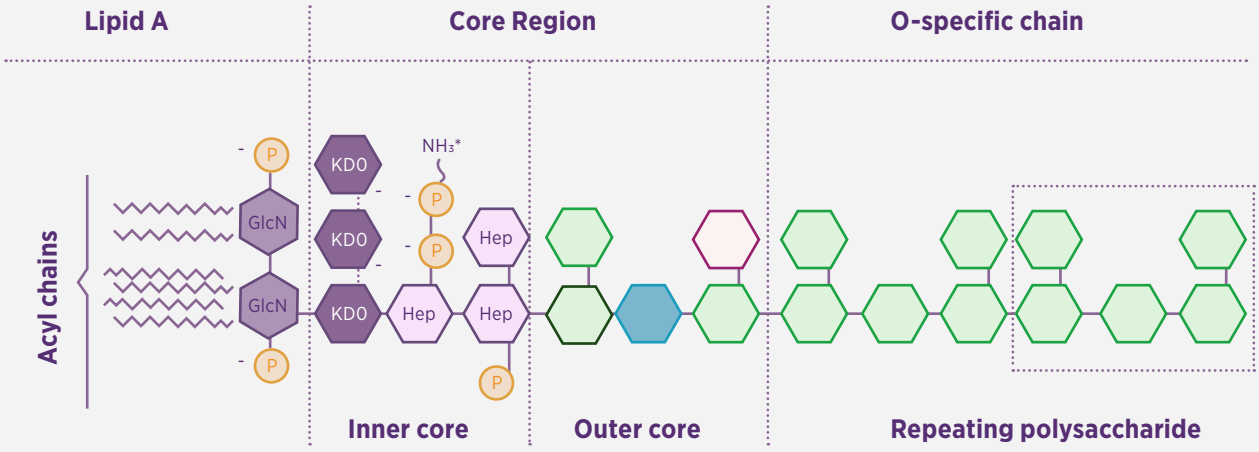


Figure 1. *Schematic structure of the LPS from E. coli (Adapted from Abate et al., Journal of Medical Microbiology, 2017).*

Endotoxins vs. Exotoxins

Both, endotoxins and exotoxins are potentially hazardous to cell cultures, animals, and humans, though they elicit their effects through different mechanisms.

EXOTOXINS

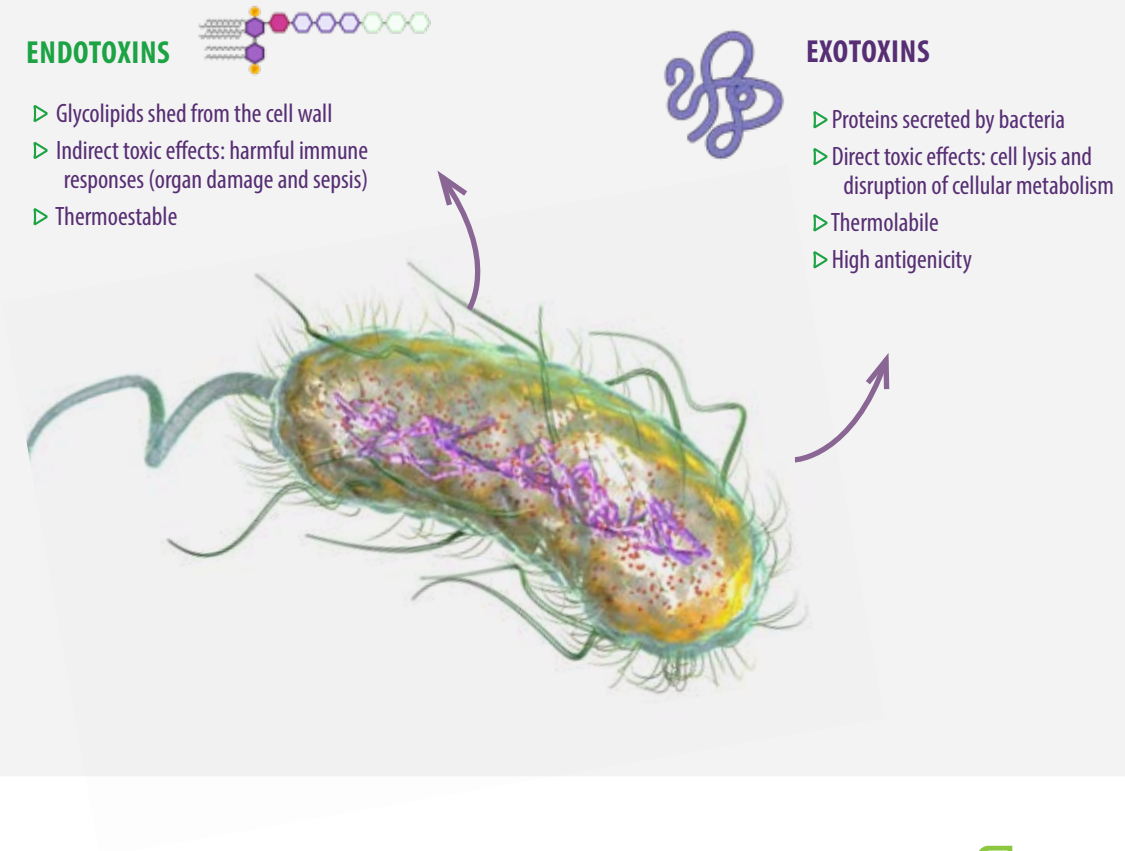
Exotoxins are potent toxic substances, usually **proteins, secreted by bacteria** and released outside the cell.

- They have **direct toxic effects** on host cells, including **cell lysis** and **disruption of cellular metabolism**.

ENDOTOXINS

Endotoxins are **glycolipid components of the cell wall** of the bacteria and are not secreted but **shed during bacterial growth or lysis**.

- They are not directly toxic to cells but can induce **harmful immune** responses when they enter the circulation that may result in organ damage and death (sepsis).



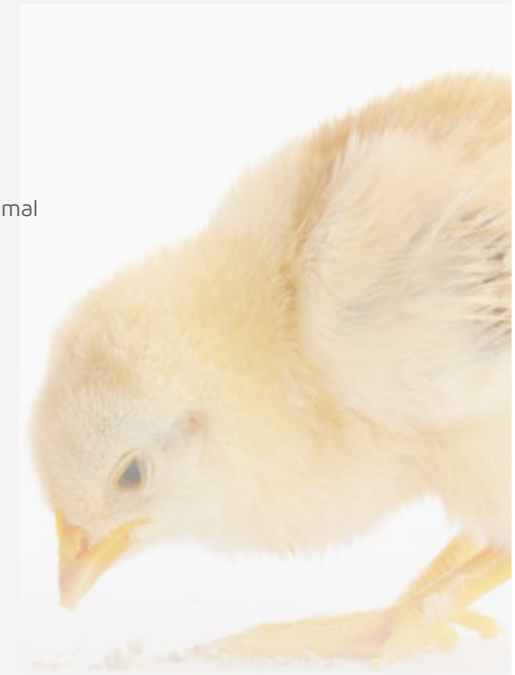
Exotoxins are usually destroyed by heat whereas **endotoxins are heat stable** (high temperatures ~200°C for 60 minutes may destroy endotoxin structures and must be used to 'depyrogenate' the equipment).

Exotoxins are highly antigenic and they elicit a **stronger antibody response** than endotoxins. In fact, **denatured exotoxins** (toxoids) may be used to develop **vaccines**.

Endotoxins in animal feed

Endotoxins are present everywhere in the environment: in the air, the water, soil and in the gastrointestinal tract of animals.

Protecting all livestock from their harmful effects should be a priority for everyone from feed to farm. Via feed, **livestock are constantly exposed to endotoxin-containing Gram-negative bacteria.**



- In 1993, the FDA tested for the presence of *Salmonella enterica* in animal feed samples from 78 rendering plants that produced animal protein. ***S. enterica* was detected in 56% of the 101-animal protein—based samples and 36% of the 50-vegetable protein—based samples**.
- In 1994, the FDA tested 89 finished feed samples collected from feed mills and from farms where animal feed is mixed and found that **25% of the samples were contaminated with *S. enterica*.**
- Exposure to endotoxin in feed is associated with disease.

Common symptoms in livestock are **sudden death or nervous symptoms**, such as blunting, staggering, ataxia, subcutaneous oedema particularly in nose, ears, eyelids, and larynx (squeaky voice). Therefore, **measures should be taken to effectively remove or detoxify endotoxins before they can reach the blood circulation.**

Transfer of endotoxins

In production animals **the gastrointestinal tract is the main risk site where endotoxins can be transferred from the lumen into the bloodstream**, where they exert their harmful effects.

In healthy animals, the permeability of the gut is tightly controlled.

The **gut barrier** is composed of **gut epithelial cells (enterocytes)** which are connected by **tight junction proteins**.

- **This keeps endotoxins on the luminal side of the gut where they are not toxic to the animal.**

The immune system constantly 'senses' these endotoxins by **specific receptors (TLR-4) present on the membrane of enterocytes**.

- In conditions of good gut health, this is one of the mechanisms that help **protect the animals against the constant threat of endotoxins**.

Several external factors are known to increase gut permeability. Furthermore, a disrupted gut barrier is characterised by a **higher number of TLR-4 receptors** due to excessive triggering of the immune system. Altogether, this promotes **leakage of endotoxins into the bloodstream (Figure 2)**.

In ruminants, the translocation of endotoxins into the blood circulation can also take place across the rumen epithelium. This epithelium has a multi-layer structure and is covered by keratinised cells, which act as a protective barrier.

Moreover, TLR-4 receptors are also present to protect the animal against endotoxins. However, a low ruminal pH and a high osmolality reduce the epithelial barrier function and increase endotoxin translocation from the rumen into the blood. Moreover, physiological stress and exposure to materials which damage the epithelial layer (such as mycotoxins) increase gut permeability and allow translocation of large amounts of LPS.

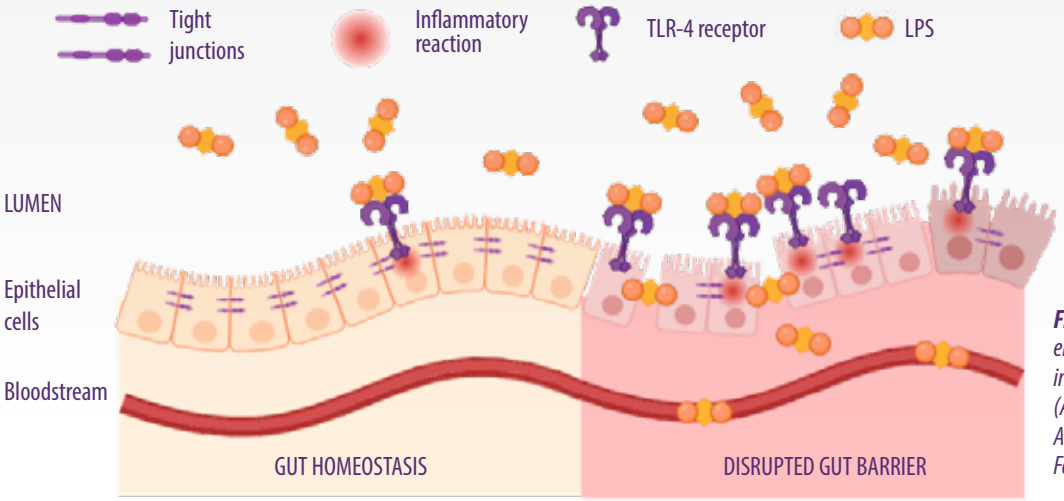


Figure 2. *Transfer of endotoxins from the lumen into the bloodstream (Adapted from Endotoxins – A constant threat - All About Feed)*

LPS modes of action

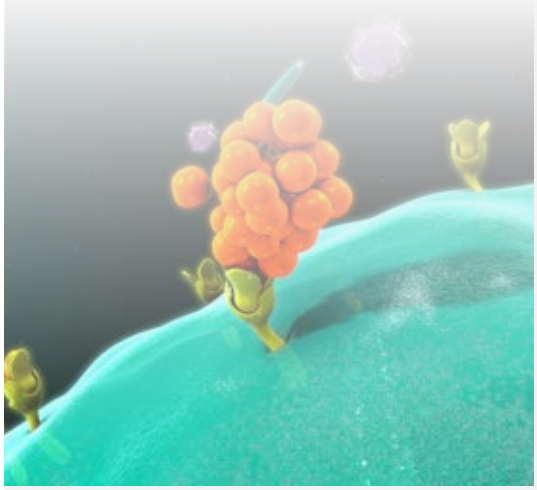
The physiological consequences of the **presence of endotoxins in the bloodstream (endotoxemia)** are quite complex.

LPS act as a signal of infection, stimulating a **vigorous inflammatory response** which leads to the pathological effects of this molecule. Responses to LPS include:

Stimulation of TLR-4

The **stimulation of the LPS receptor TLR-4** (Toll-like receptor 4) present on immune cells such as **monocytes and macrophages**, leads to **secretion of critical pro-inflammatory cytokines**, primarily interleukin (IL) IL-1β, IL-6, IL-8, and **tumor necrotic factor (TNF) α and β**.

In the blood, LPS can form a **complex with the LPS binding protein (LBP)** which facilitates transfer of LPS to TLR4 to initiate the signalling cascade leading to **activation of inflammatory genes**.



Activation of the Complement system

LPS activates the Complement system – a series of plasma proteins that normally protect the host from pathogens by inducing an i**nflammatory response, binding to pathogens to aid their removal by phagocytes and forming a membrane attack complex (MAC)**, which perforates the membranes of pathogenic bacteria.

Dysregulated activation of Complement, by large amounts of LPS, will cause **damage to host cells and tissues**.

Expression of TF

LPS stimulates expression of tissue factor (TF) on immune cells. TF forms a **highly procoagulant complex with activated coagulation factor VII (FVIIa)**, leading to the formation of **fibrin blood clots**. Fibrin can also initiate inflammation which can lead to activation of coagulation.

Excessive activation of the coagulation system, coupled with LPS-induced suppression of anticoagulant mechanisms, leads to **life-threatening disseminated intravascular coagulation (DIC)**.

Synthesis of proinflammatory factors

LPS stimulates the synthesis of lipid mediators from membrane phospholipids including **PAF** (platelet-activating factor). PAF has many **pro-inflammatory effects on blood vessels and airway cells**.

Endotoxemia is associated with the development of **sepsis – a dysregulated systemic inflammatory and immune response to microbial invasion** that produces organ injury and carries a **high mortality**.

Consequences of endotoxemia include hypotension, metabolic acidosis, hemoconcentration, intestinal hemorrhage, fever, activations of neutrophils and endothelial cells, and predisposition to thrombosis.

Sepsis may manifest as:

- Reduction in blood pressure and increased heart rate (hemodynamic alterations)
- Abnormalities in body temperature
- Progressive hypoperfusion at the level of the microvascular system
- Hypoxic damage to susceptible cells
- Quantitative changes in blood levels of leukocytes and platelets
- Disseminated intravascular coagulation (DIC)
- Multi-organ failure
- Death of animal

Sepsis with a profound hypotension is described as septic shock and carries a high mortality.

ENDOTOXIN TOLERANCE

If an animal is continuously challenged with endotoxins, a condition of **endotoxin tolerance** and **compensatory anti-inflammatory response syndrome (CARS)** can appear.

This consists of a state of hypo-responsiveness characterized **by reduced capacity of immune cells to respond to inflammatory stimuli**, particularly those initiated by LPS. **Such depression of the immune system leaves the animal exposed to the actual pathogens**.

In **endotoxin tolerance**, extensive reprogramming termed as "innate immune training" leads to **modifications of the intracellular components of TLR signalling** and also to **alterations in extracellular soluble mediators**.

How can we mitigate the effects of endotoxins?

Different strategies can be implemented to minimise the occurrence and effects of endotoxins in animals:

1. **Vaccines with endotoxin components.** Lipid A has been used experimentally to obtain vaccines for protection. However, due to structural variability, **protection from antibodies cannot be guaranteed.** In addition, the big drawback is the **high cost of production.**

2. To prevent the impact of endotoxins when applying **bacterins**, it is critical to follow the recommendations on the application of vaccines issued by the manufacturing companies.

3. **Use of mycotoxin binders (Inorganic or organic binders).** The use of mycotoxin binders to bind endotoxins is reported, however the binders should be tested for adsorption of endotoxins before use.

An additional benefit of these binders is the **adsorption of mycotoxins** that are frequently present in feed and can **contribute to the alteration of the gut permeability of livestock allowing translocation of LPS**. Trials correlating the use of mycotoxin binders with disease rates and outcomes should also be carried out.

Adsorption of endotoxins using Minazel, Minazel Plus and MycoRaid

Minazel, **Minazel Plus** and **MycoRaid** are mycotoxin remediation products produced and sold globally by **PATENT CO.**, Serbia.

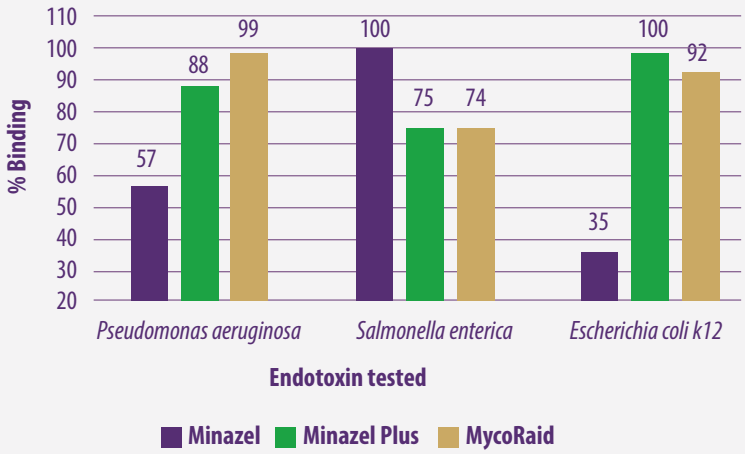
Minazel is a product based on **Clinoptilolite** (zeolite).

Minazel Plus is produced by **Organically modified Clinoptilolite** (zeolite).

MycoRaid is a product that contains **mineral premixes, yeast cell wall, *Bacillus sp.*, and herbal extract.**

Molendotech Ltd., UK carried out studies to determine the **LPS binding capacity of Minazel, Minazel Plus and MycoRaid** in pH 6.5 buffer using 10 mg/ml binder concentration and results are shown in ***Figure 3***.

Figure 3. *Endotoxins binding with Minazel, Minazel Plus and MycoRaid.*



Protocol

- Endotoxins: **LPS from *E. coli* O111:B4, *S. enterica typhimurium*, *P. aeruginosa*, *E. coli* K-12** at final concentrations of 1ng/ml were incubated with the binder at 10mg/ml in pH 6.5 buffer.
- Endotoxins from each source were incubated with buffer at pH 6.5 to obtain a **binder-free control sample**.
- Endotoxins from each source were incubated with Polymyxin B in buffer at pH6.5 to obtain a **positive binding control sample**.
- Samples were incubated for 1 hour at 37°C, centrifuged and the **endotoxin concentration in the supernatant was determined by LAL** (Limulus Amebocyte Lysate) **assay**.
- Any endotoxin present in the binder alone or buffers were also determined by LAL assay.

Results

- **MycoRaid** can bind **99% of *Pseudomonas aeruginosa* endotoxins, 92% of *E. coli* K1-2 and 74% of *Salmonella enterica* endotoxins.**
- **Minazel** can bind **57% of *Pseudomonas aeruginosa* endotoxins, 35% of E. coli K-12 and 100%** of ***Salmonella enterica*** endotoxins.
- **Minazel Plus** can bind **more than 88% of *Pseudomonas aeruginosa* endotoxins, 100% of *E. coli* K-12** and **75% of *Salmonella enterica* endotoxins.**

Endotoxins can cause significant health problems in animals, promoted by feed contaminants and physiological stresses. This leads to low food to weight conversion, often culling and loss of income.

Removal of endotoxin improves feed quality and improves animal welfare. Therefore, **use of mycotoxin binders with effective adsorption rates against endotoxins is recommended.**