ENDOTOXINS IN COMMERCIAL POULTRY OPERATIONS

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The importance of endotoxins in poultry production is a subject of much interest in some regions, as a result of marketing in the recent years of several products having positive effects in birds, while other works showed adverse effects in other species.

Something we quickly detected when reviewing some of these reports indicating effectiveness in birds, is the absence of measurements indicating that endotoxins were actually present in the animals evaluated. Therefore, it is worth asking,

how can we associate improvements in production parameters when using these additives if they do not detect the presence, absence or decrease in the level of endotoxins in treated animals?

We fully understand that a major limitation in detecting whether these toxins are present in animals is being able to use reliable laboratory tests to measure their concentration in blood, urine or feces.

- So far, the evidence available is not very accurate and the interpretation of the results tends to confuse researchers.
- In the last decade, we have participated in the evaluation of the results of field tests carried out in pig farms in the European Union, where endotoxins were suspected of causing production deficiencies and where a commercial product was tried to try to reduce or neutralize its effects.
- Although a favorable (statistically significant) effect was reported in various production parameters when using the additive, it was not clearly established that the changes were caused by its inclusion in the feed.

What are endotoxins?

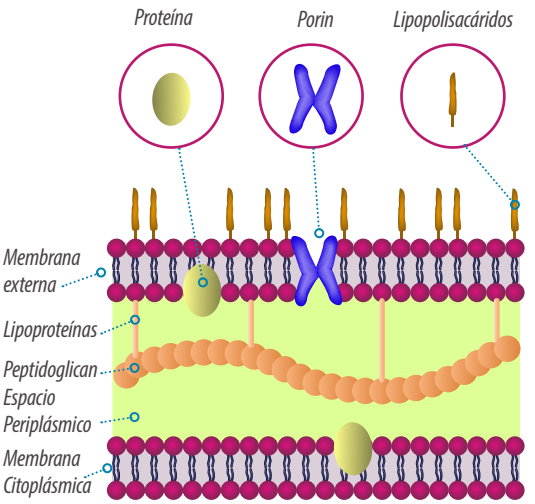
They are structural components of Gram Negative type bacteria, regardless of pathogenic or non-pathogenic, consisting of protein material and fats that form the outer membrane of the bacterial cell wall.

Gram Negative bacteria have a cell envelope that contains three layers or membranes:

- Cytoplasmic membrane (interior),
- Peptidoglycan membrane or R layer
- Outer membrane.

Since Gram Positive bacteria do not have an outer membrane, they do not contain endotoxins.

Figura 1. *Esquema estructural de una bacteria Gram Negativo.*



In bacteriology, the term is used for a complex containing lipopolysaccharides (LPS) associated with the outer membrane of bacteria such as *Eschericia coli,* Salmonella, Pseudomonas and Pasteurella, among others.

The most important bacteria from the point of view of animal health that contains endotoxins, belong to the group of Enterobacteriaceae and in many cases are part of the normal intestinal microbial flora of birds and mammals (including humans).

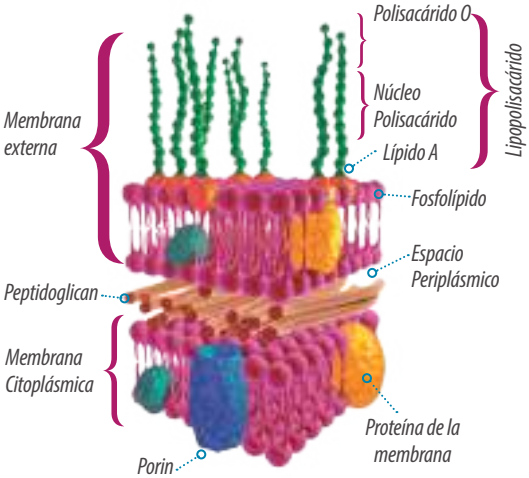
LPS are composed of **three elements: Lipid A, nucleus and O antigen.**

Lipid A is a hydrophobic structure, does not mix or repel water, which is associated with toxicity and acts as an anchor when bacteria invade the cells of the affected animal. Lipid A is always the same regardless of the bacteria, even though endotoxins differ from each other. This factor explains why endotoxins from different bacteria cause the same type of damage in the host.

The second component of LPS is the **nucleus**, an oligosaccharide.

The third component is the **O antigen**, a hydrophilic structure (mixed with water) and stimulates the immune response, being associated with the pathogenesis of some infections.

Figura 2. *Estructuras importantes en una bacteria Gram Negativo.*



How are endotoxins released?
Two mechanisms have been detected:

1 Production during the initial phase of bacterial growth, both in the laboratory (*in vitro*) and in animals (*in vivo*).

- Through this mechanism, minimal amounts of soluble endotoxins are released.
- It is important to emphasize that for this growth and release to occur, a liquid medium is needed.
- Once they are released from the cell wall, an immune response is initiated that depends on the type and concentration of LPS, duration of exposure, host's genetics and the presence of clinical signs of simultaneous viral or bacterial infections.

2 Product of the destruction (lysis) of bacteria by the effect of the immune system or antimicrobial agents.

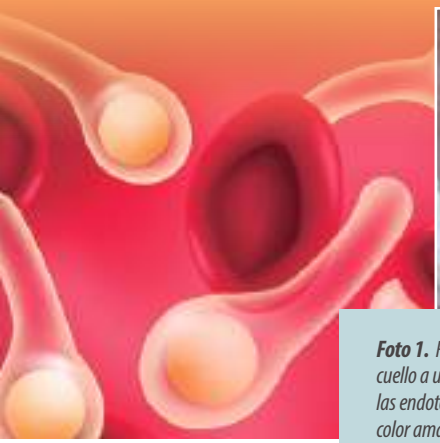
- Since the intestines are loaded with Gram Negative bacteria, they represent the largest source of endotoxins in animals.
- Its elimination in the feces allows them to combine with food and form a bio-aerosol that can stimulate an inflammatory response in the respiratory tract.
- In experimental tests, when performing intra-tracheal inoculation of endotoxins in broilers, hypertension of the lungs occurs and it is speculated that it may play an important role in the development of ascites syndrome.
- To emphasize the great dissemination capacity of this bio-aerosol in commercial poultry production, the same type of endotoxin present in birds has been detected in the blood of personnel working on farms.

When animals are subjected to an excessive level of stress, as in the case of farrowing in mammals, the level of free endotoxins in the body increases. Once the balance established in the intestinal microbial flora is lost (dysbacteriosis or dysbiosis), the final result of these conditions is the development of Salmonellosis and/or Colibacillosis. **In other words, the absence of some beneficial bacteria, for example, Lactobacillus, will allow pathogenic bacteria to grow in the intestines.**

It is important to differentiate endotoxins from exotoxins. The latter are produced by both Gram Negative and Positive bacteria, with a highly specific effect on the host.

pathology

Unlike endotoxins, exotoxins are secreted by bacteria and small amounts are very lethal, as in the case of botulism and secretions can be converted to toxoids, as in the case of the tetanus vaccine, produced by ***Clostridium tetani.***



Can endotoxins cause harm to commercial birds?

For decades, deleterious effects have been observed when injecting bacterins prepared with Gram-negative pathogens. Scientific publications have shown that by injecting small concentrations of endotoxins from Pasteurella multocida into broilers, the clinical signs of acute cholera can be reproduced, without the need to challenge the whole bacteria.

The best example of this type of damage at a commercial level is the effect of the application of the bacterin against Avian Cholera, which is characterized by causing decline and fever in vaccinated birds (pullets) in growth.

On the other hand, the reactions at the application site are very important due to seizures at the slaughterhouse level, especially when using oil emulsions. A recommendation by the biological and genetic lines companies that illustrates the importance of endotoxins when vaccinating, is to **keep vaccines at an average temperature of approximately 37 ºC because overheating causes the release of endotoxins contained in the suspension vaccine.** The release of endotoxins present in the vaccine causes severe reactions and mortality and can cause a hemorrhagic syndrome.

Foto 1. *Reacción localizada en el lugar de aplicación de una bacterina inyectada en el cuello a una pollona comercial de 12 semanas de edad y ocasionada probablemente por las endotoxinas (LPS). La foto fue tomada a las 18 semanas y muestra material caseoso de color amarillento de origen bacteriano por la contaminación de la aguja o la jeringuilla.*

How are endotoxins prevented?

The most important thing is to determine if they are actually causing any harm to birds, before investing in prevention. If a real effect is determined, the following strategies can be used.

1. **Vaccines with endotoxin segments.** .... Lipid A has been used experimentally to obtain protection. The big drawback is the high cost of production.

2. **Use of mycotoxin binders mixed with the feed (Clay or organic binder).** In *in-vitro* tests, about 90% of adsorption capacity of endotoxins is reported, however this does not mean they necessarily work when used in animal feed.

   Several scientific tests carried out on pigs in the United States have demonstrated the efficacy of some clays in neutralizing the negative effects, diarrhea and poor productive parameters, by challenging experimentally with pathogenic strains of E. coli. Although endotoxins are not measured in these reports, it is speculated that part of the effectiveness of these products is a consequence of neutralizing the endotoxins released by the bacteria used in the challenge.

3. If we want 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**.

In conclusion, despite the fact that the adverse effects caused by endotoxins in pigs and commercial-type dairy cattle seem to be more defined, in the case of commercial birds their impact is not categorically supported.

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