The gut is the first line of defence against endotoxins

Endotoxins, also known as lipopolysaccharides (LPS), are cell wall components of Gram negative bacteria such as E. coli and salmonella. They are released upon bacterial replication or lysis. They are toxic to production animals and can significantly reduce immune status and zootechnical performances.

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Farm animals are continuously exposed to LPS throughout their lives. The main route for exposure is the gastrointestinal tract. The intestine is the key link between present endotoxins and their detrimental effects on the animal.

This challenge to intestinal health will predispose the animal to intestinal infections and impair efficient digestion and absorption of nutrients with the associated effect on animal productivity. So it all starts with the gut and its intestinal lining.

When passing by the intestinal lining endotoxins can be transported transcellular or paracellular through the enterocytes.

Enterocytes contain different surface proteins, such as efflux proteins that are responsible for pumping toxins back to the lumen or tight junction proteins that glue enterocytes together. When tight junction proteins are affected by toxins more translocation of bacteria and toxins from the lumen towards the lamina propria will take place, resulting in an increased immune response and a state of inflammation also referred to as leaky gut.

When more toxins and bacteria are passing the gut barrier they will trigger pro-inflammatory cytokines and will eventually lead to inflammation, which again will result in more damage to the cell lining and leakage, which is a vicious circle.

So, in conclusion, endotoxins increase permeability of the intestinal epithelial layer, resulting in excessive and uncontrolled leakage of foreign material into the animals leading to inflammatory and altered immune responses and eventually impairing or resulting in impaired growth and performance.

Decreased resistance or increased permeability not only leads to increased translocation of bacteria, but also to nutrient leakage. This gives Gram negative bacteria more proteins to grow and hence produce more endotoxins.

All of these factors together affect animal productivity and producer profitability, and should always be taken into account when assessing the cost effectiveness of counteracting toxins.

	Contamination level			Elitox
	LPS	AFLA	T2	
Control	-	-	-	-
Challenged	100µl LPS from E.coli O111:B4	315.8ppb	858.6ppb	-
Challenged + Elitox	100µl LPS from E. coli O111:B4	315.8ppb	858.6ppb	2kg/T

Table 1. Overview of trial setup. Mycotoxin contamination levels were determined by HPLC on feed batches at start of the trial.

Inflammation not only compromises the integrity of the intestine, but also has an energy and nutrient cost.

When comparing LPS to other infections or diseases leading to growth losses, endotoxins lead to the third biggest loss, next to digestive bacterial infections and mycotoxins, mainly due to decreased feed efficiency and reallocated energy away from growth towards maintenance and immunity.

How can Elitox prevent endotoxicosis

The gut is the first line of defence against endotoxins and, if compromised due to nutrition, stress or metabolic state, endotoxin transport can increase, for example, mycotoxins, inflammation, heat stress etc. Elitox has been developed over the last 18 years to successfully eliminate toxins present in the gut and provide the immune support needed to restore gut damage. Many in vivo challenge trials have shown its effectiveness not only against mycotoxins, but also against LPS challenge.

The efficacy of Elitox against endotoxins was recently evaluated in two trials in vitro and in vivo in collaboration with the university of Melbourne, Faculty of Veterinary and Agricultural Science and with Universidade Federal do Parana, Veterinary Clinical Pathology Laboratory.

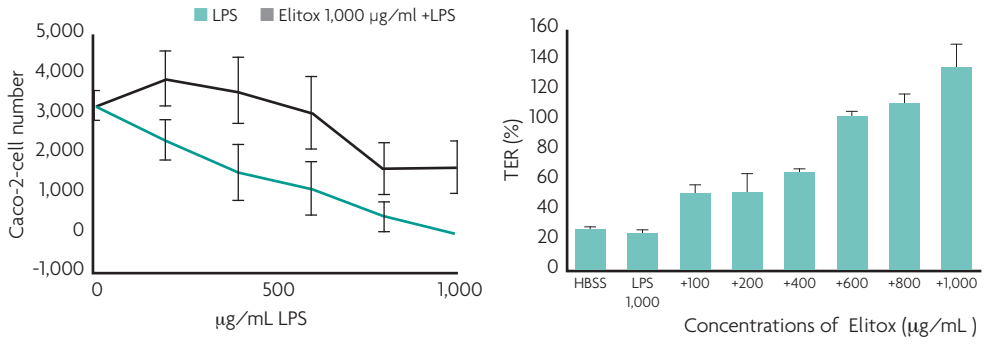
In the in vitro study the effect of Elitox was assessed on bacterial LPS mediated inflammation and toxicity on Caco-2-cells. These intestinal cell lines have been extensively used over the last 20 years as a model of the intestinal barrier. When cultured in a plate they lead to the formation of a monolayer of cells, expressing several morphological and functional characteristics of the mature enterocyte.

Cytotoxicity of LPS was evaluated in the presence and absence of Elitox in well plates in which LPS was added to the medium for two days. Elitox prevented LPS mediated toxicity in these Caco-2-cells, resulting in significant higher cell numbers as demonstrated in Fig. 1.

Moreover, trans epithelial resistance (TER) evaluated over the monolayer of Caco-2 cells in hanging baskets was evaluated. Elitox clearly increased mucosal resistance and

Continued on page 29

Fig 1. (a) Caco-2-cell toxicity by LPS addition to the medium in the absence and the presence of 1000µg/mL Elitox and (b) Transepithelial resistance of enterocyte monolayer challenged with LPS in hanging baskets, where increasing concentrations of Elitox were added (from 100-1000µg/mL) and compared to a negative control with Hanks balanced salt solution (HBSS).



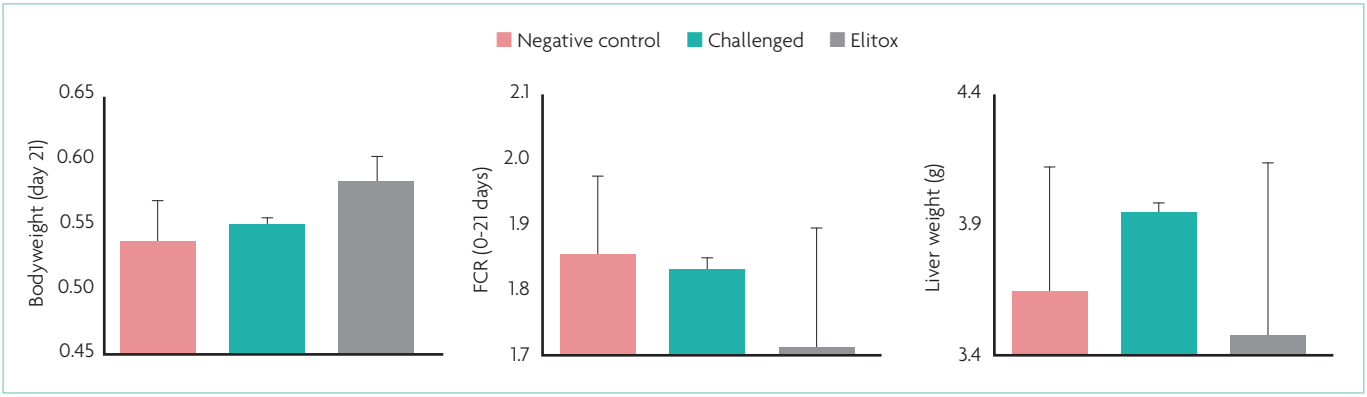


Fig. 2. Effect of combined endo- and mycotoxin challenge on zootechnical parameters. Body weight (BW), feed conversion ratio (FCR) and the weight of the liver determined during a trial period of 21 days.

Continued from page 27
hence prevents permeability. From this preliminary in vitro research it could be concluded that Elitox not only protects enterocytes against toxins from fungi but also reduces the toxic effect induced by endotoxins from bacteria (LPS) and protects the intestinal barrier.

This gave us confidence to set up an in vivo experiment where we challenged broilers with LPS in combination with mycotoxins (aflatoxin and T2-toxin) to evaluate the immune modulating and hepato-protective effect of Elitox in our own in vivo research facilities located in Brazil, in collaboration with the university of Parana for blood analysis and Imunova Biological Analysis Company for immune response.

Trial results

In total, 84 day-old male Cobb broiler chicks, vaccinated against infectious bronchitis at hatch, were individually weighed and divided into three groups, each composed of four replicates of seven animals with similar initial body weight.

The replicates were randomly distributed into separate metal cages of 0.5m². A starter corn/soybean meal based diet was formulated. Purified aflatoxin and T2-toxin (from Fermentek) were added to treatment two and three (Table 1). Body weight and feed consumption was controlled at cage level weekly to calculate FCR.

Two animals per cage were injected with 100µl LPS from E. coli 0111:B4 (10mg LPS dissolved in 100ml phosphate buffered solution) 24 hours before blood sampling at day 21. Blood was sampled exactly 24 hours post LPS injection and submitted to flow cytometry to determine circulating lymphocytes (at Imunova Biological Analysis Company) and inflammation responses, measuring acute phase proteins (ovotransferrin) and pro-inflammatory cytokines (IL-6).

These same animals were used for immunological organ sampling (liver, spleen and bursa). Due to the very low number of animals per cage at the end of the trial (day 21) no significant differences and due to the LPS trigger high variations were observed on zootechnical performance.

However, Elitox treated animals had numerically the highest body weight, highest daily weight gain and lowest feed conversion (Fig. 2).

Weight of the immunological organs was affected. Contamination leads to heavier immune organs, whereas Elitox statistically reduced liver weight (Fig. 2). The spleen is the organ where all lymphocyte proliferate. B-lymphocytes further differentiate in the bursa, whereas T-lymphocytes further differentiate in the thymus. Changes in the weight of these immune organs can be used as an indicator for triggered immunity.

Circulating immune cells were determined by flow cytometry. Helper T-lymphocytes originating from the mucosa were significantly increased in contaminated animals, whereas reduced to control levels when Elitox was supplemented (Fig. 3).

Cytotoxic and non-activated helper T-lymphocytes were not significantly affected, although numerically higher in contaminated animals. This finding was supported by the increased pro-inflammatory cytokine, Interleucin-6 in contaminated birds (Fig. 4).

Mycotoxins trigger an immune response which costs energy without added value.

On the other hand, the first line defence cells, such as monocytes, which cost little energy are decreased in contaminated animals. When supplementing Elitox the first line immune responsiveness increased. Acute phase response, another first line defence mechanism, was also significantly increased in Elitox supplemented animals, supporting the increased monocyte response. This type of non-specific immune response is important for fast growing and short living animals without reallocating too much energy away from growth towards immunity.

Conclusion

Endotoxins can elicit strong immune responses, weakening immune systems and impairing performance.

In the body of the animal, endotoxins cause an inflammatory cascade that increases maintenance requirements, resulting in less energy available for growth. Moreover they also impair feed efficiency. From in vitro research on Caco-2 cells, as a model for intestinal lining, it was shown that Elitox protects the enterocytes and increases resistance, decreasing permeability or gut leakage. In vivo research on LPS challenged broilers, supported the theory that LPS weakens immune systems and impairs performance as clearly shown in varying immune parameters. Elitox was found to be very effective in restoring appropriate immune responsiveness and strengthening the first line defence of challenged animals. ■

References are available from the author on request

Fig. 3. Effect of combined endo- and mycotoxin challenge on the immune response. Circulating lymphocytes were differentiated by their surface markers (CD4/CD8) by flow cytometry and expressed relative to the total peripheral blood mononuclear cell (PBMC).

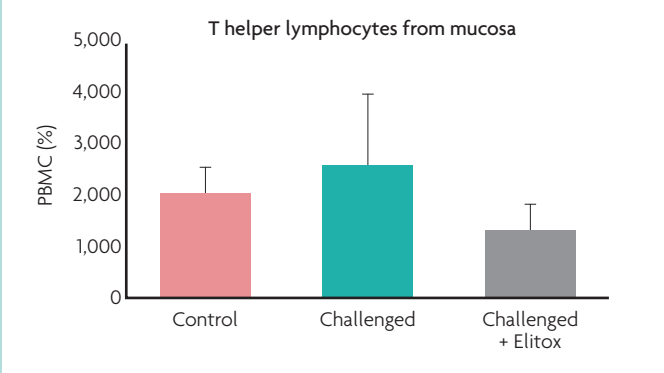


Fig. 4. Interleukin 6 (IL-6) was determined by a commercial ELISA kit.