Newsletter

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> SUMMARY

The issue of endotoxins is a well-known problem worldwide. Unfortunately, it is almost impossible to perform in vivo experiments in this field due to the difficulty of artificial challenge of the animals by adding endotoxins in the feed, which often leads to failure.
One of the methods for controlling the negative impact of endotoxins in animals is to use an appropriate material to adsorb endotoxins in the gastrointestinal tract, leading to reduced bioavailability and increased excretion of the adsorbent-endotoxin complex. Similar to aflatoxins, endotoxins can be bound by selected adsorbent materials.
However, the choice of the correct substances against endotoxins entails more than just performing adsorption tests, as the counteraction of endotoxins goes beyond their binding ability. The procedure BIOMIN involves a multi-step approach to selecting the appropriate binding material followed by testing Mycofix® product line for its anti-inflammatory properties.
In the following newsletter, you will find out more about effective counteraction of endotoxins and preventive action for successful endotoxin risk management.

Competence Center
Mycotoxin Risk Management

Mycofix® product line

Concept for counteracting endotoxins in ruminant-related diseases

Endotoxins are part of the outer membrane of the cell wall of Gram-negative bacteria (e.g. *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, amongst others) and are independent of whether the organisms are pathogenic or not. By definition, an endotoxin is a toxin that is not secreted in soluble form by live bacteria, but is instead a structural component in the bacteria which is released mainly when bacteria are lysed. Released endotoxins reach the circulation and, in healthy animals, are bound to different serum constituents and lipoproteins and delivered to the liver where they are neutralized (liver cells). Endotoxins are stored in a body's fat tissue or eliminated through the mammary gland, gut and lungs. In the case of damage in the rumen barrier and raised rumen permeability (acidosis), the liver has to combat higher load of endotoxins at the same time.
We know that laminitis is one of many endotoxin-related diseases and that all trigger factors of laminitis are not yet fully understood. Endotoxins together with other toxins like mycotoxins decrease the blood supply of lamella tissue, and increase the blood pressure in the hoof/claw. Related to the direct effects of endotoxins is the increased production of specific cell-activated cytokines (e.g. TNF-α, IL-6), triggered by endotoxins. This starts the inflammation process, leading to the occurrence of laminitis.

Lab tests and pre-tests/Adsorption of endotoxins

As Polymyxin B (PMB) is able to bind endotoxins, it is a very useful tool for endotoxin research. Polymyxin antibiotics are relatively neurotoxic and nephrotoxic and are usually used only as a last resort if modern antibiotics are ineffective or are contraindicated. We included PMB into our endotoxin adsorption test as positive control to show how the Mycofix® product line is effective in endotoxin binding.

Results of the endotoxin binding assay are shown in *Figure 1*. Even at low concentrations of 0.1 %, 0.05 % and 0.025 %, the used bentonite, a part of the Mycofix® product line, adsorbed 99.46 %, 91.01 % and 75.33 % of endotoxins (initial concentration was 7.5 EU/ml), respectively. Results are given in comparison with Polymyxin B (PMB) which was almost 100 % effective in endotoxin binding.

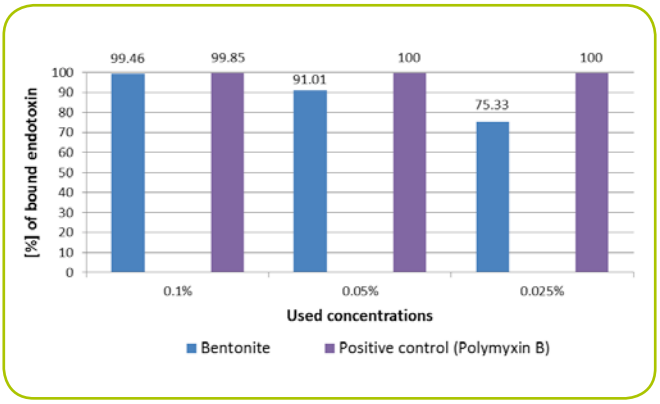

Figure 1. *Percentage of endotoxins adsorbed by the Mycofix® product line in comparison with Polymyxin B*

Immuno-modulatory effects of the Mycofix® product line

The anti-inflammatory assay was performed to test the influence of the Mycofix® product line on the capacity of the murine macrophage cell line to produce anti- or pro-inflammatory cytokines. Murine macrophage cell lines are susceptible to endotoxins (LPS) and are a well-established test system.

The Mycofix® product line offers a complex-strategy solution for the counteraction of endotoxin effects. Adsorption components enable the binding of endotoxins. Mycofix® product line stimulates the production of the anti-inflammatory cytokine (IL-10) and inhibit the production of pro-inflammatory cytokines (IL-6 and TNF-α) (*Figure 2*).

During pathogenesis of laminitis pro-inflammatory cytokines (e.g. TNF-α, IL-6) and the activated enzymes weaken or even destroy the connective tissue between the lamella and pedal bone, which might be one of the most serious triggers of laminitis. To support this hypothesis, we performed an *ex vivo* laminitis test model.

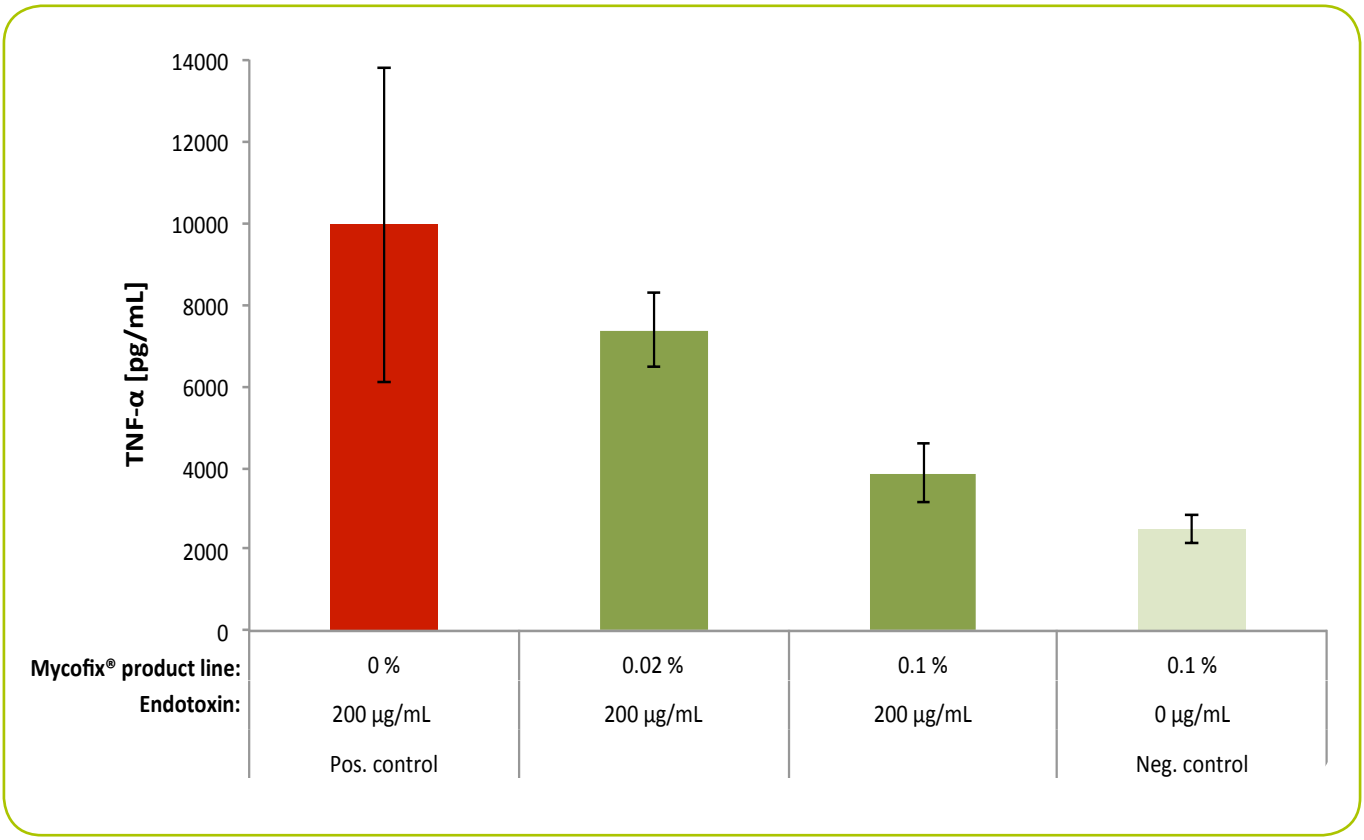

Figure 2. *Decreased production of pro-inflammatory cytokine (TNF-α) in the presence of Mycofix® product line in comparison with increased production of TNF-α in the presence of endotoxin (200 µg/ml).*

Ex vivo laminitis model

As already mentioned, the challenge of animals by endotoxins is very time and cost-intensive, associated with pain and stress for the animal, and leading finally to unsuccessful outcomes. Therefore an *ex vivo* model of laminitis is very useful. In this model, living tissue from the hoof or claw, taken from the slaughter house, is cultivated *in vitro*. After cultivation with possible trigger factors (endotoxins, mycotoxins etc.), it is tested for possible separation of the connective tissue from the lamella and the amount of force needed (*Figure 3*). Trigger factors as well as potential substances for reversing the negative effect of the trigger factors can be tested in this system.

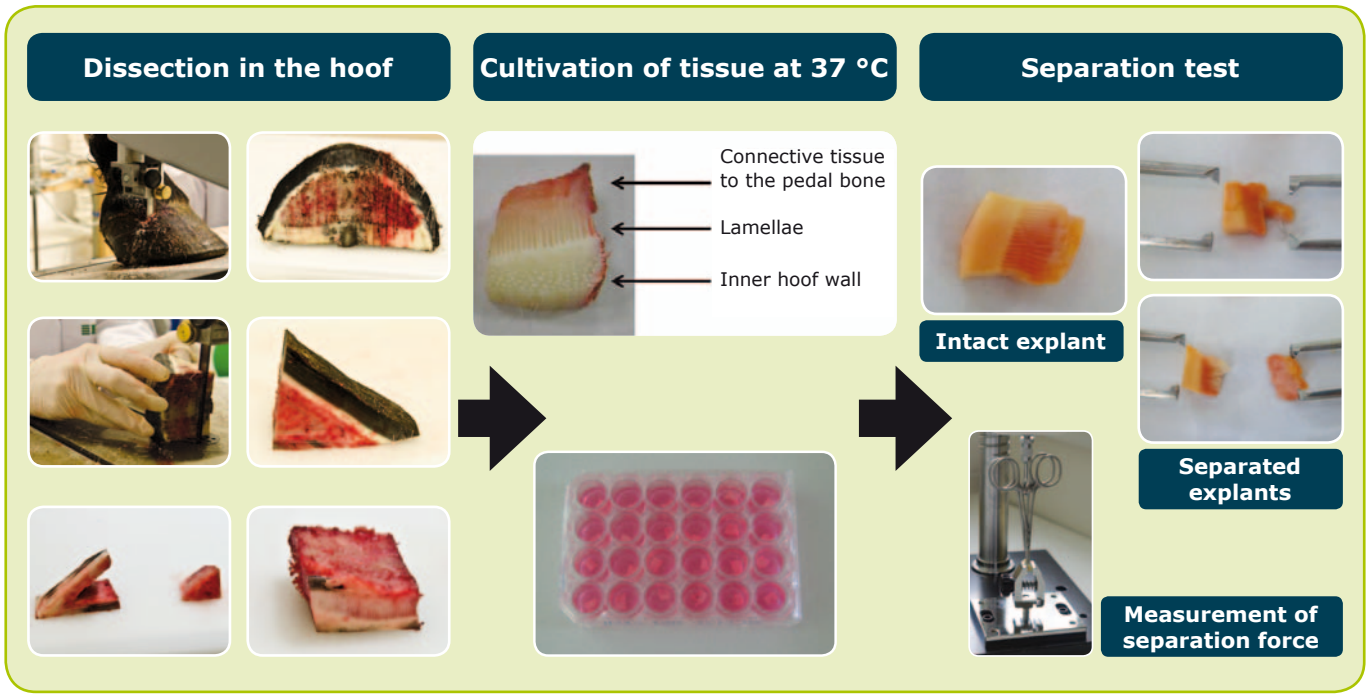

Figure 3. *Overview of the process of the equine and bovine ex vivo laminitis model, which is used to test potential trigger factors and substances which can inhibit this effect*

Results

Concentration of 20 µg/ml endotoxins separated all explants. At a concentration of 5 µg and 10 µg/ml LPS, separation of 33 % and 66 % of the explants could be detected (*Figure 4*).

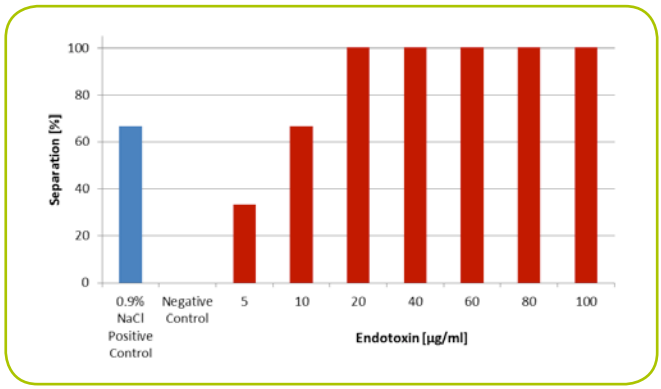

Figure 4. *Concentration-dependent separation of explants after 24 hours incubation with 0.9 % NaCl solution (positive control) or endotoxins in percentage*

Endotoxins showed a concentration-dependent lamellar separation in claw explants. Therefore, the same *ex vivo* model was used to test the influence of the mycotoxins fumonisin B_1 (FUM) on hoof explants. Explants were incubated with different concentrations of FB_1 (0.5 – 10 ppm). Incubation of explants with fumonisin B_1 led to lamellar separation (*Figure 5*).

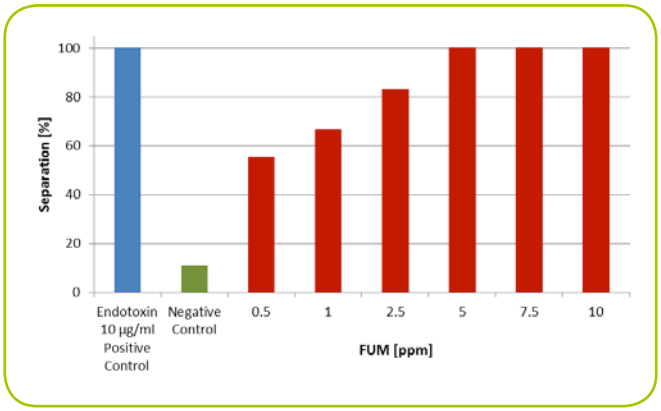

Figure 5. *Concentration-dependent separation of explants after 24 hours incubation with 10 µg/ml endotoxin (positive control) and different concentrations of fumonisin B_1 (FUM)*

Ex vivo laminitis model experiments already confirmed that endotoxins, as well as fumonisin B_1, have a negative influence on the hoof tissue. With the successful development of this model, we are only one step away from proving the *ex vivo* efficacy of the Mycofix® product line which will give us a good basis to perform reliable *in vivo* experiments.

Testimonial

600 dairy cows farm with an average milk production of 36 kg milk/day, Wisconsin, USA

The farm is using Mycofix® Plus for eight months. Improved reproductive performance and hoof health were reported after this period of time. Hoof trimmer and veterinarian reported fewer foot problems (hairy foot warts) than at any other time before and very few foot problems in general.

Conclusion

Lameness is an increasing problem associated with higher production, more intensive feeding and confined conditions. Laminitis is a multifactorial disease whose origin is not clearly understood (a possible etiology was provided in BIOMIN Newsletter, Vol. 11, No. 124 Special edition). Laminitis results in claw horn lesions that significantly affect the cow's well-being, productivity and longevity. Despite the multifactorial etiology of laminitis and many possible reasons for this disease, an effective endotoxin and mycotoxin risk management approved by field observations seems to be one of the promising approaches to decreasing its incidence in practice.

References available upon request

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