Technical Article

Detoxifying activity of Detoxa Plus®

The following work is a summary and interpretation of the scientific article published in the journal **TOXINS NUMBER 8**, on October 15, 2019.

"Enzyme Degradation Reagents Effectively Remove Mycotoxins Deoxynivalenol and Zearalenone from Pig and Poultry Artificial Digestive Juices"

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Mycotoxins are toxic and complicated secondary active biological metabolites produced by filamentous fungal species, mainly *Aspergillus*, *Fusarium* and *Penicillium* [1]. When animals consume feeds contaminated with mycotoxins, they suffer from a series of toxic effects, such as decreased feed intake, reduced body weight gain, diarrhea, vomiting, as well as liver and kidney pulmonary edema [2]. Many different strategies, such as thermal inactivation, irradiation, physical dilution, and anti-mycotoxin products (adsorbents or elimination reagents), are used in feed mills and farms to reduce mycotoxin concentration of feedstuffs and feeds [3].

The addition of anti-mycotoxin products in feed is currently an effective strategy for detoxifying animal feeds. There are two major types of anti-mycotoxin feed additives, i.e., adsorbents and elimination reagents. Mycotoxin adsorbents work by preventing the absorption of mycotoxins by the gastrointestinal tract of the animal by adsorbing the toxins to their surfaces. Adsorbents are either inorganic (e.g., bentonites and hydrated sodium calcium aluminosilicate) or organic products (yeast cell membrane) [4,5]. Enzyme reagents (EDRs) aim to alter the toxic chemical structure of the mycotoxins and further reduce their toxicity. They generally take the forms of the whole bacterium, yeast cultures or specifically extracted components, such as enzymes [6].

Mycotoxins are classified into polar and non-polar categories, according to their chemical structures [7]. Polar mycotoxins, such as aflatoxin B1 and fumonisin B1, are more easily adsorbed by adsorbents than non-polar mycotoxins. On the other hand, the solubility, molecular weight of mycotoxins – in case of ionized compounds – charge distribution and dissociation constants play a critical role of mycotoxin adsorbent ability [8]. aflatoxin B1 has a higher adsorption rate than fumonisin B1. Therefore, aflatoxin B1 is commonly used as a target for evaluating the mycotoxin removability of adsorbents [9].

Most mycotoxin adsorbents have demonstrated lower performance (or even lack of performance) in terms of the ability to adsorb non-polar mycotoxins like DON and ZEN. On the other hand, biological elimination of DON and ZEN by commercial EDRs is a suitable way to control associated toxicities as supported in many previous studies [10,11].

Removability is commonly used as a standard measurement for assessing the removal efficiency of mycotoxins, but to date a robust method for the evaluation of mycotoxin removers is currently unavailable. the mycotoxin removability can be determined by using both *in vitro* and *in vivo* assays. However, the ultimate goal of *in vitro* study is aimed to replace *in vivo* experiments in general. Therefore, the conditions of *in vitro* experiments should be tightly controlled and well-designed to closely resemble the natural environments of the target animal species, in turn, leading to high reproducibility of research data [12]

In the present trial, an in vitro model that resembles the conditions of the gastrointestinal tract of the bird is used. The model replicates the passage time of feed in each section of the intestine (crop, proventriculus, gizzard and intestine), the pH of each section of the intestine, the temperature, the intestinal motility and the presence of digestive enzymes characteristic of the animal. In this way, a model that fits, with great satisfaction, to the conditions in vivo was achieved.

For the study a dose of DON challenge was used according to the guidelines of China Hygienic Standard for Feed (GB13078-2017) and FDA regulations, resulting in a dose of 5,000 ppb [13].

The anti-mycotoxin additives evaluated, shown in Table 2, were selected based on those with the highest market prevalence. Products were used at the doses recommended by the manufacturers.

BRAND/COMPANY	DOSAGE (KG/TN)
Detoxa Plus® New/ BV Science*	0,5
Detoxa Plus®/BV Science	1
International enzymatic competitor (EDR1)	1
Asian enzymatic competitor (EDR2)	1
Asian enzymatic competitor (EDR3)	1
Mineral adsorbent competitor (MAD1)	2
Mineral adsorbent competitor (MAD2)	2

Table 2. EDR: Enzymatic Inactivators * Product under development, not available for sale. For detailed information please see the full paper.

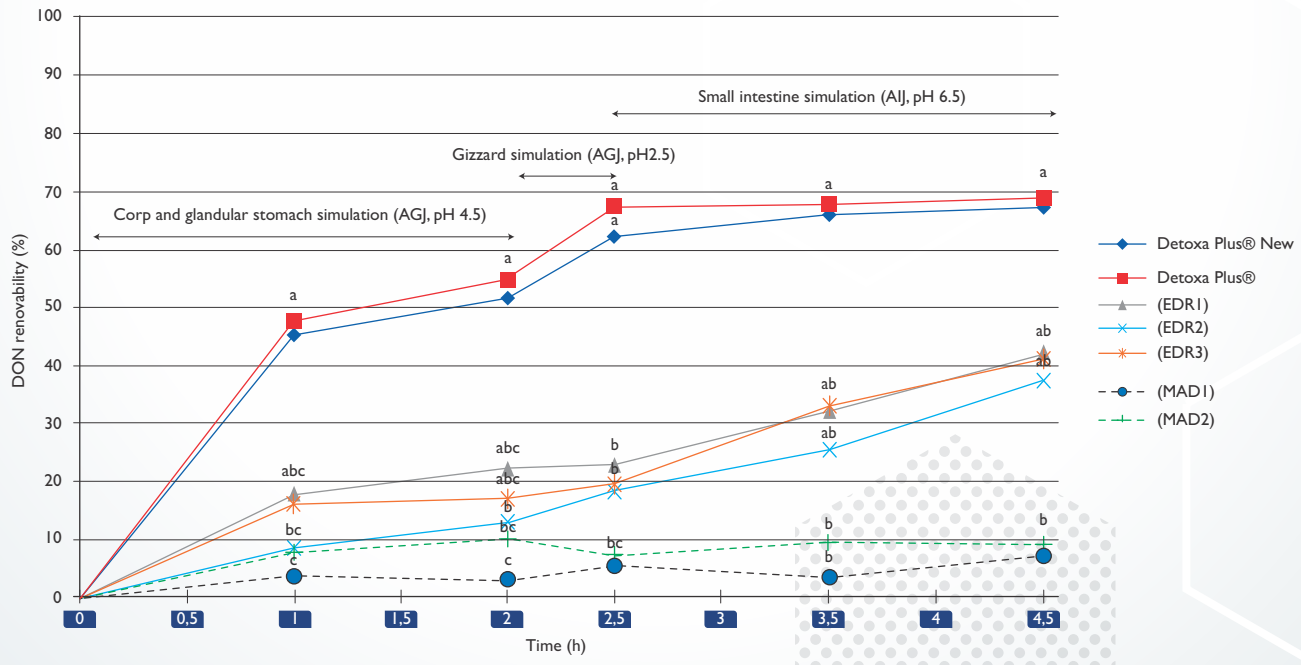
HPLC was used for the evaluation since it is considered the most accurate method to evaluate mycotoxins.

The initial dose was 5,000 ppb of DON, this dose is well above the maximum recommended limits for the species (TABLE 3).

	DON (ppb)
CHIKS	200
BROILERS	500
FINISH FEED	1000
LAYERS	1000
BREEDERS	1000

Table 3. Source: Mycotoxicological analysis laboratory (https://www.lamic.ufsm.br/site/legislacoes/legislacao-brasil)

Graph 1 shows the ability to remove DON as a function of time along the in vitro model of the gastrointestinal tract of a bird.



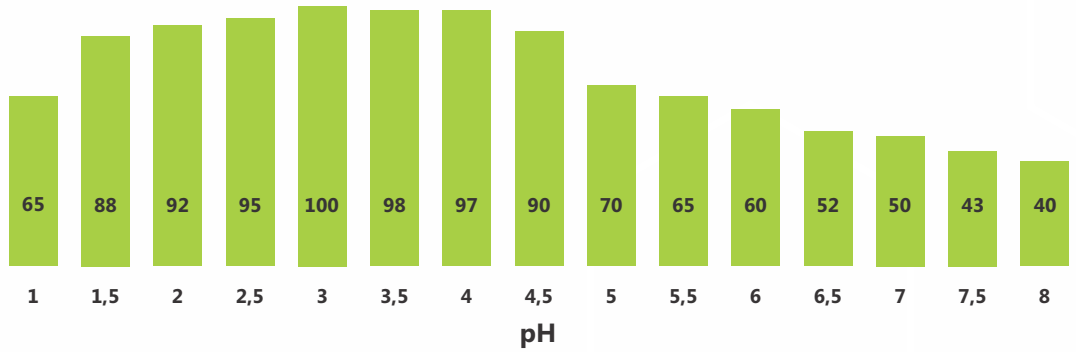
Graph 1. Removal of DON (initial 5,000 ppb) over 4.5 hours at different pH levels with the presence of enzymatic inactivators (solid line) or adsorbents (dotted line).
AGJ: artificial gastric juice,
AIJ: artificial intestinal juice.
EDR: enzymatic inactivator.
Different letters in the same column indicate significant differences between treatments with p <0.05.

Discussion

When we evaluate the first 2 hours, equivalent to crop and proventriculus we can see how **Detoxa Plus®** and **Detoxa Plus®** New (product under development, not available for sale) have a significantly higher elimination capacity (50% and 52% respectively) to the rest of the enzyme inactivators (22% for EDR1, 13% for EDR2 and 17% for EDR3) and the sequestrants (3% for MAD1 and 7% for MAD2). When the pH drops further, due to the passage of the solution to the gizzard, we find an even greater increase in the removal capacity of **Detoxa Plus®** and **Detoxa Plus® New** (67% and 62% respectively), while the rest of removers do not present significant changes.

This ability to eliminate mycotoxins in the first portions of the gastrointestinal tract is specific to **Detoxa Plus®**, since, unlike the rest, the enzymes present in the product have their peak of enzymatic activity at acidic pH.

Percentage of enzymatic activity at different pH



When the solution reaches the intestines, and an increase in pH is generated (from 2.5 to 6.5), we show how the biotransformation capacity of **Detoxa Plus®** is diminished, while the rest of the enzyme inactivators increase significantly. This variation occurs, since the enzymes of the rest of the products evaluated, have their peak activity at a pH closer to neutrality. On the other hand, the activity of the sequestrants does not vary, demonstrating that their adsorption capacity is not influenced by the pH.

Finally, after the total transit of the solution through the animal model (4.5 hours), a TOTAL removal capacity of DON from **Detoxa Plus®** and **Detoxa Plus® New** of (69% and 68% respectively) was obtained while that the rest of the enzyme inactivators had an action of 42% (EDR1), 41% (EDR2) and 38% (EDR3). In turn, the adsorbents had a DON removal capacity of 7% and 9% for MAD1 and MAD2 respectively.

The difference in total elimination of DON between Detoxa Plus® and the rest of the products is due to the fact that the enzymes of **Detoxa Plus®** begin to work at acidic pH, in the first portions of the gastrointestinal tract, which allows a longer time of total action. The rest of the products increase their speed of action within the intestine, where passage time is short.

Conclusion

The present work demonstrates that enzymatic inactivators are a robust option for the elimination of DON in the *in vitro* model and within these, the **Detoxa Plus®** and **Detoxa Plus® New** are the ones with the greatest elimination capacity. In turn, under the same challenge conditions, the other products tested were not able to eliminate DON.

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