

## Efficacy and safety testing of mycotoxin-detoxifying agents in broilers following EFSA guidelines

Osselaere A.<sup>1</sup>, Watteyn A.<sup>1</sup>, Devreese M.<sup>1</sup>, Vandenbroucke V.<sup>1</sup>, Goossens J.<sup>1</sup>, Eeckhout M.<sup>2</sup>, De Saeger S.<sup>3</sup>, De Baere S.<sup>1</sup>, De Backer P.<sup>1</sup>, Croubels S.<sup>1</sup>

1. Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.

2. Ghent University College, Schoonmeerstraat 52, 9000 Gent, Belgium.

3. Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Gent, Belgium.

Mycotoxin-detoxifying agents are added to the feed to reduce mycotoxicoses in poultry. A lot of feed additives are on the market, but only few trials were performed to study their *in vivo* efficacy. In 2009 the European Commission introduced a new functional group within the existing category of technological additives: 'substances for reduction of the contamination of feed by mycotoxins'. This additional group can be divided in two groups, namely adsorbing and biotransforming agents. We performed an *in vivo* experiment according the recent guidelines of the European Food Safety Authority (EFSA, 2010). Forty-eight 1-d-old broiler chicks were randomly assigned to 1 of 8 dietary treatment groups after one week of acclimatization. The table below illustrates the different diets for the groups:

group	DON ( $\mu\text{g}/\text{kg}$ diet)	detoxifying agent
group 1	/	/
group 2	2439 $\pm$ 700 (artificially contaminated)	/
group 3	2439 $\pm$ 700 (artificially contaminated)	adsorbing agent
group 4	2439 $\pm$ 700 (artificially contaminated)	biotransforming agent
group 5	/	adsorbing agent
group 6	7540 $\pm$ 2200 (naturally contaminated)	/
group 7	7540 $\pm$ 2200 (naturally contaminated)	adsorbing agent
group 8	7540 $\pm$ 2200 (naturally contaminated)	biotransforming agent

During this study, zootechnical parameters, such as feed intake and weight gain were measured. No adverse effects in the broilers were noted by inclusion of DON with or without the detoxifying agents in the diets, compared to control diet. As reported by EFSA these parameters can not be used solely for demonstration of efficacy of mycotoxin-detoxifying agents. Following EFSA guidelines the most relevant endpoint for testing additives against DON is measuring DON and its metabolites in blood serum. Blood samples were taken after 7, 14 and 21 days of feeding. The levels of DON and its metabolites in plasma and tissues were determined by a validated LC-MS/MS method (De Baere et al., 2011). No DON or metabolites were detected in the plasma of group 2, 3 or 4. For the animals of groups 6, 7 and 8, DON, DOM-1 and 3-ADON were detected and the concentrations decreased with time. After three weeks of feeding no groups showed levels above the limit of detection. These results suggest that *in vivo* trials for testing detoxifying agents against DON in broilers have some limitations when plasma concentrations are used as endpoint. Feeding concentrations of DON below the maximum authorized level of 5000  $\mu\text{g}/\text{kg}$  results in DON levels below the limit of detection, resulting in no assessment of the efficacy of mycotoxin-detoxifying agents.

The European Commission also asks to take the presence of possible interactions with veterinary medicinal products into account. An extra trial was therefore performed including three groups of broilers: blank feed, blank feed with an adsorbing agent and blank feed supplemented with a biotransforming agent. The antibiotics oxytetracycline (100 mg/kg B.W.) and amoxicillin (20 mg/kg B.W.) were given to the animals by an oral bolus after 4 weeks of feeding. A kinetic study was performed and the concentrations of both antibiotics in plasma were determined by a validated LC-MS/MS method. The results will be presented at the conference. **Acknowledgements:** The authors would like to thank L. Goethals, V. Hautekiet and SANLUC International NV for their financial support. The assistance of J. Lambrecht and A. Van den Bussche is gratefully appreciated.

### References

EFSA, 2010. Statement on the establishment of guidelines for the assessment of additives from the functional group 'substances for reduction of the contamination of feed by mycotoxins'. The EFSA Journal 2010; 8(7) :1693.

De Baere S, Goossens J, Osselaere A, Devreese M, Vandenbroucke V, De Backer P, Croubels S, 2011. Quantitative determination of type-A and type-B trichothecenes and their related compounds in animal body fluids using LC-MS/MS detection, submitted.