VALIDATED MULTI-MYCOTOXIN ANALYSIS USING DRIED BLOOD SPOTS FOR PIGS AND BROILER CHICKENS APPLIED IN A SCREENING AND TOXICOKINETIC STUDY.

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The contamination of animal feed with mycotoxins leads to deleterious effect on animal health and performance. A first step in reducing these effects is measuring the *in vivo* exposure of the animals. Currently, assessing mycotoxin exposure is mostly done in feed. However, to measure individual exposure and eliminate the problems of hotspots in feed, the mycotoxins need to be determined in biological fluids such as blood. The use of dried blood spots (DBS) as sampling technique has some major advantages. First of all, only a small amount of blood is needed to detect the mycotoxins in comparison with conventional plasma collection. Moreover, the filter cards are easy to store and transport which is especially interesting when sampling animals in the field and on farm.

The presented method aims to detect mycotoxins and their phase I and II metabolites in DBS obtained from the ear of pigs and the leg vein of broiler chickens using LC-MS/MS and LC-HRMS equipment. The detected mycotoxins and metabolites belong to the regulated groups: aflatoxins, ochratoxin A, Fusarium mycotoxins (deoxynivalenol, zearalenone, T2 toxin, fumonisins) and to the group of emerging mycotoxins with high prevalence in feed: enniatins, beauvericin and tenuazonic acid. Whole blood of pigs was collected via the jugular vein and 60 µL was spotted on a filter card. Next to these artificial dried blood spots needed to obtain calibration curves and quality control samples, DBS were also made by direct spotting of blood form the ear of the pig tonto the card. After 12h drying, the card can be stored in -20 °C. Next, for extraction a vast area is punched out of the card and extracted with water/acetone/acetonitrile (30/35/35). The extract was dried and reconstituted in 60 µL of water/methanol/formic acid (60/40/0.1). This methods was in house validated according to European and International guidelines.

The method was applied in a pilot toxicokinetic study in one pig and one broiler chicken as proof-of-concept study. The pig and the broiler chicken got an oral bolus of deoxynivalenol, aflatoxin B1 and ochratoxin A. DBS and blood were taken before administration (0 min) and 5, 10, 20, 30, 45, 60, 90 min and 2, 3 and 4h after administration. The whole blood was centrifuged to obtain plasma. Furthermore, a screening study was performed in sows. Therefore, farms with postpartum problems (e.g. PDS) that might be related to mycotoxins were selected. In each farm 12 DBS were obtained from the ear and stored in -20°C until analysis. The preliminary data of this screening study and the results of the toxicokinetic study will be presented at the conference.