

Penetration of the antibiotic fosfomicin into swine intestinal mucosa colonized with *Lawsonia intracellularis*

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Introduction/Objective:

Fosfomicin (FOS) is an antibiotic (ATB) used for the treatment of lung and enteric infections of pigs. Intracellular fluids of enterocytes can act as biophase for *Lawsonia intracellularis* (LAW), causative agent of porcine proliferative enteropathy (PPE), which presence could modify ATB penetration. The aim of this study was to determine FOS penetration into swine intestinal mucosa colonized with LAW.

Materials and Methods:

Four healthy pigs in grow-finish stage; live attenuated vaccine (Enterisol Ileitis®, Boehringer-Ingelheim); primers for LAW PCR detection (GATAATCTACCTTCGAGACGG; TGACCTCAGTGTTCAGTTATCGT, Invitrogen); calcium FOS. Explants were produced from ileum of euthanized animals. As a positive control for the PCR, LAW DNA was obtained from the vaccine. Explants (0.5 mL of vaccine; 24 h of incubation; 37°C) were incubated with 580 µg/mL of calcium FOS (0.5 to 6 h). Then, they were washed to remove extracellular FOS, deproteinized (1 mL of methanol) and sonicated (30 min) to release intracellular ATB. Tubes were centrifuged (6 min; 4°C; 10,000 rpm), supernatants were evaporated to dryness (60°C), dry extracts were dissolved in 200 µL of HPLC water and 1 mL of Folch reagent (hexane-ethanol; 1:0.2) was added for lipids removal. Samples were shaken (20 min), centrifuged (6 min), hexane phase was discarded, 40 µL of each sample were taken and carried to 800 µL with HPLC water, filtered and analyzed by HPLC MS/MS.

Results:

Intracellular concentration of FOS ranged between 3.75 and 24.81 µg/mL (Tmax: 4 h).

Discussion and Conclusions:

On previous studies on healthy swine intestinal explants, we have found that a low concentration of ATB enters into the enterocytes (5.84-12.99 µg/mL; Tmax: 2 h), which could be attributed to the soluble nature of FOS. When comparing intracellular concentrations of FOS found in explants with LAW vs. those found on healthy pigs intestinal explants, a higher proportion is present in explants with the bacteria (4%) than in those explants without LAW (2%). However, differences were not statistically significant ($p > 0.05$). Although FOS concentrations are not too high, they exceed the MIC₉₀ for *E.coli* (0.5 µg/mL) and *Salmonella* (4 µg/mL). There are no studies indicating FOS MIC₉₀ for LAW. Nevertheless, MIC₉₀ of various ATB for LAW ranges between 0.125 and 128 µg/mL. Further studies should be carried out to determine FOS MIC₉₀ for LAW to discern the usefulness of this ATB in the treatment of PPE.