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INTESTINAL CONCENTRATIONS OF ENROFLOXACIN AFTER DIFFERENT DOSAGE REGIMENS IN BROILER CHICKENS

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Introduction and Aims

The posology of veterinary antimicrobial drugs is currently determined by dose titration and confirmation studies solely monitoring clinical efficacy. These studies generally do not take into account the objective of limiting the emergence and spread of resistance. Nevertheless, resistance to antimicrobial drugs is one of the leading health concerns in human and veterinary medicine worldwide. As resistance development and selection depends on the extent of exposure to antimicrobials, it is of importance to know to which amount of drug the intestinal microflora is exposed after treatment.

The aims of present study are to: 1/ develop a LC-MS/MS method for quantitation of enrofloxacin in intestinal content
2/ assess and compare the intestinal concentration of enrofloxacin after treatment of broiler chickens with the conventional dosage regimen, after dose escalation and with different administration routes (oral vs. intramuscular)

Materials and Methods

Sample clean-up: To 1 g of intestinal content were added the internal standard (sarafloxacin), 3 mL PBS buffer and 5 mL ethylacetate. After extraction and centrifugation and evaporation to dryness, samples were resuspended in 250 µL of H_2O.
Chromatography: Zorbax® Eclipse plus C18 (100 mm x 3.0 mm i.d., d.p.: 3.5 µm) (Agilent). Mobile phases: (A) 0.1% glacial acetic acid in H_2O (B) acetonitrile.
Mass spectrometry: TSQ® Quantum Ultra triple quadrupole MS (Thermo Scientific) operated in the + ESI mode.
SRM transitions for identification and quantification (*): enrofloxacin: m/z 360.0 > 316.2*/245.1, sarafloxacin: 386.1 > 368.0/299.0*
Animal experiment: Ninety-six animals were divided in 4 groups, each allocated to a different dosage regimen of enrofloxacin (Baytril®):
Group 1: conventional treatment: 10 mg/kg BW, 5 days, oral (by oral gavage)
Group 2: 50 mg/kg BW, 5 days, oral (by oral gavage)
Group 3: 10 mg/kg BW, 5 days, intramuscular
Group 4: 50 mg/kg BW, 5 days, intramuscular
At 2 and 4 h after first administration, and 4 h after the last administration (100 h after first administration), intestinal content (ileum, cecum, colon and cloaca) was collected from eight animals/group. Samples of the eight chickens/sampling point were pooled and 1 g aliquots were extracted in triplicate for quantitative analysis.

Results and Discussion

Method validation (as described by [1], [2]): results fell within the specified ranges:
- Linearity: concentration range 0.1 – 20 µg/g, r ≥ 0.99, g ≤ 10%
- Accuracy: within -20% to +10% of theoretical concentration (0.1, 0.5 and 10 µg/g)
- Within-day precision: RSD < RSD_{max} of 15.1% (0.1 µg/g), 11.8% (0.5 µg/g), 7.5% (10 µg/g)
- Between-day precision: < RSD_{max} of 17.8% (0.5 µg/g), 11.3% (10 µg/g)
- LOQ: 0.1 µg/g
- LOD: S/N ≥ 3 (0.07 ng/g)
- Specificity: no interferences
- Matrix effect: 73.2%

Animal experiment
- Enrofloxacin concentrations in ileum, cecum, colon and cloaca are presented in Figure 2
- High concentrations (µg/g range) were observed after oral as well as intramuscular administration
- Intestinal concentrations are dose dependent
- After oral administration, a time dependent shift was observed from ileum (2 h) to more distal intestinal segments (4 and 100 h) after oral administration
- Following intramuscular administration, the highest concentrations were detected in the cloaca, most probably due to mainly urinary excretion of enrofloxacin
- Future *in vitro* experiments can be performed to evaluate the effect of the different enrofloxacin concentrations on resistance selection in the intestinal microbiota

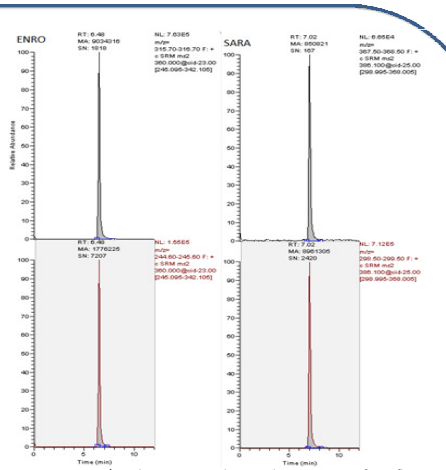

Figure 1. LC-MS/MS chromatogram showing the SRM traces of enrofloxacin (ENRO) and sarafloxacin (SARA) in standard solution (0.5 µg/mL)

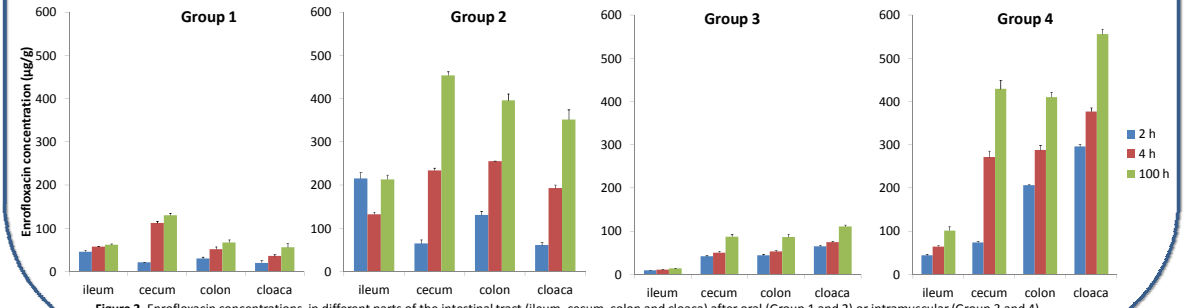

Figure 2. Enrofloxacin concentrations in different parts of the intestinal tract (ileum, cecum, colon and cloaca) after oral (Group 1 and 2) or intramuscular (Group 3 and 4) administration of 10 mg/kg BW (Group 1 and 3) or 50 mg/kg BW (Group 2 and 4) to broiler chickens

References

[1] De Baere S. *et al.* (2011). Journal of Chromatography B, 879, 2403-2415.
[2] 2002/657/EC. Official Journal of the European Union, L221.