Introduction

Coccidiosis is a disease characterised by acute invasion and destruction of the intestinal mucosa of the infected animal by protozoa of the genera *Eimeria* or *Isospora*. Due to its high reproduction potential, ability of sporulated oocysts to survive in poultry litter and its varying pathogenicity due to their prophylactic and therapeutic effects. According to Commission Regulation (EU) No 495/2011, monensin is authorised in feedstuffs at maximum doses of 125 mg/kg, and maduramicin at a maximum content of 5–6 mg/kg ([EU] No 388/2011). Despite the prescribed procedures in the production of animal feed to minimise the risk of errors and cross-contamination, the likelihood of detecting anticoccidials in food for non-target groups of animals is very high. Affinity of the drug to bind to plasma proteins, hydrophobicity and hydrophilicity, and the ability of drug transport through different types of tissues are the main characteristics affecting the distribution of the drug in the yolks or albumen of the egg (Martinez et al., 2006).

Animal treatment and sampling

Laying hens were treated with maduramicin and monensin added to feedstuffs below and at the concentration authorised for target species. Hens were divided into 4 treatment groups and fed for 14 days with medicated feed, and one control group. In the treatment groups, Groups I and II were treated for 14 days with 100% and 20% of the maximum authorised maduramicin concentrations (5 and 1 mg/kg), respectively, while Groups III and IV received 50% and 100% of the maximum dose of monensin (MC) (62.5 and 125 mg/kg). Eggs were sampled during and after treatment of hens.

Sample preparation and LC-MS/MS method

- 2 g of the homogenized egg white, egg yolk or whole egg
- Extraction of toltrazuril and its metabolite with acetonitrile
- Dissolution of evaporated supernatant with distilled water
- Evaporation and sample dilution in 250 µl methanol/water (50:50; v/v)
- Quantification was based on matrix calibration curve containing 8 blank samples spiked at 4 concentration levels

Results and discussion

- For Group 1 maximum concentration of MON in egg white was 174.2 µg/kg, and maximum concentration of MON in egg yolk was 2369.2 µg/kg.
- It can be concluded that even at high concentrations of maduramicin in poultry feed, residues of maduramicin in egg white could not be detected at significant values (Figure 1).
- Equal distribution of monensin was observed between egg yolk and egg white.
- Higher accumulation of monensin in egg white can be explained by the higher concentration of monensin added to the animal feed (Figure 2).
- Transfer factors calculated for the two experimental groups with the addition of MAD (5 and 1 mg/kg) were in the range from 0.19 to 0.36.
- Experimental groups with MON with 25 times higher concentrations in feed (125 and 62.5 mg/kg) resulted in transfer factors in the range from 0.002 to 0.0003.
- Higher concentrations of coccidostats in feed do not affect deposition of the substance in eggs.
- Concentrations of MAD and MON in eggs after treatment showed exponential decay.
- Logarithmic function of concentrations in eggs was linearly dependent with time.
- Time required for the concentration of coccidostats to decrease below the maximum permitted limits (MRLs) or below the limit of quantification (LOQ) was calculated.
- After treatment with MAD (5 and 1 mg/kg) concentration in eggs were below MRL (12 µg/kg) values 16.6 and 13.8 days after treatment (Figure 3).
- After treatment with MON (125 and 62.5 mg/kg) concentrations in eggs were below MRL (2 µg/kg) after 4.7 and 6.0 days.
- Concentrations of residues were below the LOQ values 24.4 and 22 days after treatment of MAD at concentrations of 5 mg/kg and 1 mg/kg in feed, respectively.
- If the substances dominate in egg yolk, then elimination time may take more than 10 days.
- This can be explained by the fact that the development process of egg yolk 7 begins in the liver, where the first precursors of egg yolks are generated several days prior to the laying of the egg - in comparison, egg white develops only hours before laying eggs.

Conclusion

Occurrence of residues in non-target tissues is not only affected by the degree of contamination of feed mixtures for non-target groups, but also by the pharmacokinetic properties and accumulation capability of each coccidostat.

References