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Semduramicin in eggs – The incompatibility of feed and food maximum levels

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ABSTRACT

The cross-contamination of non-target feeds with coccidiostats may result in the occurrence of their residues in food of animal origin. To assure food safety, maximum levels (ML) of coccidiostats have been set for both feed and food. However, scientific data are not available on the transfer of some coccidiostats from feed into food. This experiment was therefore designed to verify, whether the administration of compliant semduramicin-contaminated feed could cause the occurrence of violative residues of coccidiostats in eggs. The laying hens received feed containing 0.27 ± 0.034 mg/kg of semduramicin (ML = 0.25 mg/kg). Semduramicin residues were detected in whole eggs after two days of administration of semduramicin-containing diet. A plateau level was achieved (16.1 ± 5.19 μg/kg, mean ± SD) with the concentrations significantly exceeding the maximum level of semduramicin in eggs (2 μg/kg). The results of this experiment might be a signal for the revision of the ML value.

1. Introduction

Cross-contamination of non-target feeds with coccidiostats authorised as feed additives is a well-recognised problem, which may result in the occurrence of residues of these compounds in food of animal origin (Cannavan & Kennedy, 2000; Kennedy, Blanchflower, Hughes, & McCaughey, 1996). It is assumed that some level of feed contamination is unavoidable. Still, such contaminated feed should remain safe both for the animal receiving it and for the consumers of food produced from these animals.

Having the above in mind, maximum levels (MLs) for the carry-over of anticoccidials in feed were established (Anon, 2009a). The risk assessment performed by the CONTAM Panel experts of the European Food Safety Authority (EFSA) established that a level equivalent to 5% of maximum authorised concentration in feed would not be harmful for food consumers (Dorne et al., 2013). To assure even more protection, the MLs were set at 1% or 3% of maximum authorised concentration, depending on the species and categories of animals (Anon., 2009a). The level of 1% was established for animals during withdrawal periods, animals producing food in a continuous manner (laying hens and dairy cattle) and species especially susceptible to the toxicity of the specific coccidiostats.

Values of "maximum levels of coccidiostats resulting from the unavoidable carry-over of these substances in non-target feed" were set for food of animal origin (Anon, 2009b). Unfortunately, the results of experiments on animals that would characterise the transfer of the compound from feed into animal tissues and eggs were not available for every coccidiostat. In such cases, interpolation from other data was applied, which was based on the similarity of chemical properties of different compounds in the same class (especially ionophore coccidiostats) and the assumption that different species present the same pattern of ADME (adsorption, disposition, metabolism and elimination).

Recent publications concerning the administration of the ionophore coccidiostat maduramicin (MAD) to both chicken broilers (Tkáciková, Kožárová, & Máté, 2010) and laying hens (Bodi, Fry, Schafft, Lahrssen-Wiederholt, & Preiss-Weigert, 2012) have revealed that these assumptions are not necessarily confirmed with empirical data. In the experiment performed on laying hens by Bodi et al. (2012) the detected residues of MAD in eggs were much higher than expected from ML assumptions. Feeding 0.01 mg/kg maduramicin diet (20% of ML in feed) resulted in MAD concentrations up to 2.5 μg/kg in whole eggs, already exceeding the maximum level (2 μg/kg). The results of this experiment were the basis for the re-evaluation of the maximum level of MAD in eggs, which is now set at 12 μg/kg (Anon, 2012).

It is quite probable that the same situation could be observed also for semduramicin (SMD), which is chemically very close to MAD. SMD is an ionophore coccidiostat authorised for use in chickens for fattening at a concentration of 20–25 mg/kg, with five days withdrawal period (Anon, 2006). No data on the residue depletion of this anticoccidial in laying hens were available at the time of the establishment of ML. The limits of SMD in force currently are 0.25 mg/kg and 2 μg/kg in non-target feed for laying hens and eggs, respectively (Anon, 2011, 2012).

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The purpose of the study presented in this paper was to verify whether the administration of compliant SMD-contaminated feed (around ML level) to laying hens could cause the occurrence of violative residues (non-compliant results) in eggs. To the authors' best knowledge, such a study has never been published.

2. Materials and methods

2.1. Animals, study design and sample collection

The experiment was carried out in compliance with the Decision No 67/2012 of the Local Animal Experimentation Ethics Committee in Lublin. A brood of 20 laying hens was housed in a deep litter system under conventional conditions of ventilation, temperature and lighting and given water and feed *ad libitum*. During the equilibration period (seven days) hens were fed with commercial feed free from coccidiostats. Afterwards, an experimental feed containing semduramicin at a concentration close to maximum level (0.25 mg/kg) was given to the layers for 14 days. Then, for the next 14 days a withdrawal period (with a semduramicin-free diet) was applied. Eggs were collected daily during the experimental period.

2.2. The preparation and verification of experimental feed

The experimental feed was prepared in an Agropol feed mill (Motycz, Poland). The commercial complete feed for laying hens was used, which was proven to be free from semduramicin and was mixed with the feed for broiler chickens containing Aviax® premix (Phibro Animal Health, semduramicin 25 mg/kg) in the ratio of 99:1. The feed was produced in a total amount of 100 kg and divided into 25-kg sacks. Two samples of 200 g feed from each sack were then taken and analysed in duplicate using LC–MS/MS (eight samples in total for the whole batch of experimental feed). The homogeneity of the prepared feed was tested with the test of sufficient homogeneity (Thompson, Ellison, & Wood, 2006).

2.3. The determination of coccidiostats in feed

Five grams of properly ground and homogenised feed were weighed into a polypropylene centrifuge tube and spiked with internal standard (nigericin; Sigma, Saint Louis, MO). The sample was extracted with 25 mL of mixture of acetonitrile and methanol (1:1, v:v) (POCh, Gliwice, Poland). After centrifugation (3500 rpm, 10 min), an aliquot of 5 mL was purified using dispersive solid-phase extraction with 100 mg of octadecyl sorbent (J.T. Baker, Center Valley, PA). The sample was centrifuged again and an aliquot of 2.5 mL was evaporated under a stream of nitrogen at 45 °C. The sample was reconstituted in 0.5 mL of dimethylsulphoxide (Sigma) and the extract was analysed with LC–MS/MS.

The chromatography was performed on a Poroshell EC-C18, 2.7 μm, 2.1 × 150 mm column (Agilent, Santa Clara, CA) with Luna C18 precolumn (Phenomenex, Torrance, CA). The separation was performed with gradient elution of mobile phase A (acetonitrile: methanol: 0.01 M ammonium formate at pH 4.0, 60:35:5, v:v:v) and B (0.01 M ammonium formate at pH 4.0). The following gradient was applied: 0–2 min 10% A; 3–15 min 100% A; re-equilibration with initial conditions at 16 min. The mobile phase flow rate was 0.3 mL/min, the temperature of the column oven was 50 °C and injection volume was 5 μL.

The detection was performed on a triple-quadrupole API 4000 mass spectrometer (AB Sciex, Framingham, MA) with electrospray positive ionisation. The ion source parameters were nebuliser and turbo heater gas 40 psi, curtain gas 30 psi, IS voltage 5.5 kV. Two transitions were monitored for semduramicin (*m*/*z* 895.5 to *m*/*z* 833.7 and *m*/*z* 895.5 to m/*z* 851.5) and one for nigericin (*m*/*z* 747.4 to *m*/*z* 703.5).

The method was validated in-house. The results of the verification of the method for SMD at ML level are as follows: recovery 88%, intermediate inter-day precision 17.6%. The calculation of the concentration of SMD in feed was based on the matrix-matched calibration curve prepared concurrently with the analytical samples. The samples were spiked with the standard solution of semduramicin sodium (Exygen; a gift from EU-RL in Berlin) to obtain 0.05, 0.1, 0.25, 0.50 and 1.0 mg SMD/kg feed. Nigericin was used for calculation as an internal standard.

2.4. Statistical evaluation of the results of feed analyses

Homogeneity study was performed according to the procedure described in IUPAC Technical Report (Thompson et al., 2006). Eight randomly selected samples (two from each sack) were analysed in duplicate under randomised repeatability conditions. The Cochran test was used to detect analytical outliers with 95% confidence. Results were verified with the test for sufficient homogeneity. The material is deemed to be sufficiently homogenous only if:

$$s^2_{\text{sam}} \leqslant F_1\sigma^2_{\text{all}} + F_2 s^2_{\text{an}}$$

where S^2_{sam} is the between-unit (sampling) variance, S^2_{an} is the analytical variance, σ^2_{all} is the allowable variance (0.09 σ^2_p, equal to 30% of target standard deviation) and F_1, F_2 are statistical constants.

2.5. Collection of egg samples

Each day six randomly chosen eggs were broken and homogenised individually with an Ultraturrax. Such samples were kept below −18 °C for no longer than three weeks and were used for the determination of semduramicin in whole eggs.

2.6. The determination of semduramicin in eggs

The method used for the determination of semduramicin is described elsewhere (Olejnik, Szprengier-Juszkiewicz, & Jedziniak, 2010). Briefly, two grams of homogenised whole eggs were weighed into a polypropylene centrifuge tube and spiked with internal standard solution (nigericin; Sigma). The samples were extracted with acetonitrile and purified using Oasis HLB (Waters Milford, MA) solid-phase extraction cartridges. After evaporation and reconstitution of the samples, the extracts were analysed with LC–MS/MS. Chromatography was performed on a Luna Phenyl-Hexyl 3 μm, 2.0 × 150 mm column (Phenomenex) connected with a guard column. A gradient was applied with acetonitrile (phase A), methanol (phase B), and 0.01 M ammonium formate, pH 4.0 (phase C). The API 4000 mass spectrometer (AB Sciex) was operated in positive ionisation and multiple reaction monitoring mode. Two transitions were monitored for semduramicin (*m*/*z* 895.5 to *m*/*z* 833.7 and *m*/*z* 895.5 to *m*/*z* 851.5) and one for nigericin (*m*/*z* 747.4 to *m*/*z* 703.5).

For each batch of samples, the appropriate matrix-matched calibration curve from samples spiked with the standard solution of semduramicin sodium was prepared. For the analyses of samples taken from experimental days 0–4 and 21–29, a calibration curve was prepared using the standards: 0, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 μg/kg. When samples with higher concentrations were analysed (days 5–20), the calibration curve was prepared with the following concentrations: 0, 1.0, 2.5, 5.0, 10, 25 and 50 μg/kg.