Transfer of flubendazole and tylosin at cross contamination levels in the feed to egg matrices and distribution between egg yolk and egg white

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ABSTRACT Chemical residues may be present in eggs from laying hens' exposure to drugs or contaminants. These residues may pose risks to human health. In this study, laying hens received experimental feed containing flubendazole or tylosin at cross contamination levels of 2.5, 5, and 10% of the therapeutic dose. Eggs were collected daily and analysis of the whole egg, egg white, and egg yolk was performed using liquid chromatography tandem mass spectrometry. Highest concentrations of the parent molecule flubendazole, as well as the hydrolyzed and the reduced metabolite, were detected in egg yolk. Residue concentrations of the parent molecule were higher compared with those of the metabolites in all egg matrices. No tylosin residue concentrations were detected above the limit of quantification for all concentration groups and in all egg matrices. Neither molecule exceeded the set maximum residue limits.

Key words: flubendazole, tylosin, egg, feed, cross contamination

INTRODUCTION

Therapeutic doses of some veterinary drugs given to laying hens may lead to the deposition of drugs and metabolites in the eggs laid. To ensure consumer safety, withdrawal periods have been set, and only eggs laid after the withdrawal period may be used for consumption (Hekman and Schefferlie, 2011). Residues in eggs may occur from illegal or extra-label use of drugs, the use of unintentionally cross contaminated feed, or the use of mislabeled feed (Donoghue et al., 1997). Regardless of adherence to good manufacturing practice guidelines, contamination of nonmedicated feed by residues of antimicrobials or coccidiostats may occur during several steps of the production process. Besides the production lines in the multiproduct plants of the feed industry, contamination may also occur during storage at the feed mills, transport to farms, and at the farm (Segato et al., 2011).

In Belgium, flubendazole is registered as a premix, oral powder in feed or emulsion in drinking water for use in poultry with a withdrawal time of 0 d in eggs.

Tylosin is available as an oral powder administered in drinking water or feed or as premix. Only the formulation in the drinking water is registered for use in laying hens, with an egg withdrawal time of 0 d. The other formulations are not allowed for use in laying hens that produce eggs for human consumption (BCFI, 2011). Flubendazole, a benzimidazole anthelmintic, and tylosin, a macrolide antibiotic, have flubendazole and tylosin A as marker residue and a maximum residue limit (**MRL**) of 400 µg/kg and 200 µg/kg in eggs, respectively (EMEA, 2000; EMEA, 2006). In the United States, an MRL of 0.2 mg/kg is set in eggs for tylosin, which is regarded to be a negligible residue (FAS online, 2011).

Egg white and egg yolk are formed at different stages in egg production. The development of egg white takes approximately 10 h, whereas 2 to 3 h is needed for the deposition of egg white around the yolk. The formation of egg yolk takes 8 to 11 d; yolk components (predominantly lipoproteins) are formed in the liver and transported via the blood to the ovary-containing follicles in which the yolk is deposited. The majority of yolk formation occurs during the last 2 wk before ovulation (Donoghue et al., 1997; Kan and Petz, 2000; Hekman and Schefferlie, 2011). Residue levels in egg white reflect the plasma levels, whereas residues in yolk reflect the plasma levels during the 10 d of the rapid growth

phase of the follicles (Kan and Petz, 2000). The concentration of a drug is determined by the compounds pharmacokinetic behavior, which in turn is a reflection of animal physiology (organ perfusion, renal and hepatic function, membrane permeability, tissue composition, and tissue pH) and the drug's physicochemical properties (Martinez, 1998a), such as molecular weight, lipid solubility, and acid dissociation constant, as well as binding to plasma proteins. These physicochemical properties largely determine a drug's pharmacokinetics. For example, as molecular weight increases, the compound's ability to diffuse through any liquid medium decreases. The lipophilicity of a drug, expressed as the octanol/water partition coefficient (**log P**), is positively correlated with the ability to cross biological membranes. The acid dissociation constant (**pKa**) represents the pH at which an equal proportion of the drug exists in its ionized and unionized forms, and theoretically only unionized, nonpolar drugs penetrate biological membranes mainly by passive diffusion. Plasma protein binding determines the drug's availability to other compartments, as only the unbound drug molecules are distributed to the tissues (Martinez, 1998b; Kan and Petz, 2000; Kan, 2003).

In this study, we have determined 1) whether providing feed containing flubendazole or tylosin at cross contamination levels to laying hens leads to the presence of residues in egg matrices thus presenting a risk for food safety, 2) the preference of both molecules for either egg white or egg yolk and the recommended matrix for sampling to ensure food safety, and 3) whether testing for metabolites should be included in routine analysis.

MATERIALS AND METHODS

Premix, Reagents, and Standards

The premixes containing flubendazole or tylosin were Flubenol 5% premix and Tylan 250 vet premix. These premixes were kindly provided by Janssen Pharmaceutica (Beerse, Belgium) and Elanco Animal Health (Hampshire, England), respectively. Analytical standards of flubendazole, H-flubendazole, R-flubendazole, and tylosin A were obtained from Sigma (Bornem, Belgium), Witega (Berlin, Germany), Janssen (Beerse, Belgium), and Biovet (Peshtera, Bulgaria), respectively. The internal standards, D_3 flubendazole and spiramycine, were purchased from Witega and Sigma. The reagents K_2CO_3 and HCl 37% for flubendazole analysis and acetic acid 100% for tylosin analysis came from Merck (Darmstadt, Germany). An ultra-turrax at 135,000 to 205,000 rpm (Ika-werke, Yellow-line DI25, Staufen, Germany) was used to homogenize the egg matrices.

Preparation of the Experimental Feed and Bird Experiments

The feed was prepared at the Institute of Agricultural and Fishery Research (**ILVO**)'s Animal Sciences Unit.

Animal experiments were performed at ILVO's Animal Sciences Unit following the recommendations for euthanasia of experimental animals and under supervision of ILVO's ethical committee (EC. No 2010/133 and 2010/139).

Flubendazole and tylosin were incorporated in the feed at cross contamination levels of 2.5, 5, and 10% of the maximum allowed dose; that is, 30 mg/kg and 100 mg/kg for flubendazole and tylosin, respectively. After a first mix of the premix with a small amount of blank feed (Varimixer Bear, Dehaeck construct, Belgium), this amount was added to the remaining blank feed in a custom-made feed mixer constructed by Silobouw (Zulte, Belgium). The final feed was mixed for 30 min at 35 rpm with a switch of direction every 5 min to optimize the mixing process. Three rinsing batches were carried out between the different experimental feeds and 2 samples were analyzed from each rinsing batch to exclude carry-over. Ten dynamic samples were taken at the top (n = 3), the middle (n = 4), and the bottom (n = 3) of each prepared experimental feed to determine the achieved concentration.

The laying hens, medium ISA-Brown, were housed under conventional conditions of ventilation, temperature, and lightning and were vaccinated at 18 wk of age. The animals were given free access to water and feed. General and individual health as well as the feed and water supply, ventilation, and temperature (20°C) were monitored daily. Each concentration group included 18 laying hens housed in 2 enriched cages of 9 laying hens. After an adaptation period of 10 d (blank feed), the animals received the experimental feed (0, 2.5, 5, and 10% groups) for 14 d, followed by a 17-d depletion period (blank feed). Eggs were collected daily, and egg weight and number of eggs were noted. Ten whole eggs were homogenized for analysis of the whole egg. The remaining eggs were separated for analysis of egg white and egg yolk, and all samples were stored at −18°C. For all concentration groups, the whole egg was analyzed every 2 d during the treatment period and daily during the depletion period. The egg white and egg yolk analyses were performed for all concentration groups during the treatment period (d 5–14) and depletion period (d 16–17).

Sample Preparation for the Experimental Feed

Flubendazole and its metabolites and tylosin A were analyzed by the Department Santé of the Centre d'Economie Rurale and at Ghent University's Department of Pharmacology, Toxicology, and Biochemistry of the Faculty of Veterinary Medicine, respectively.

For flubendazole analysis, 3 g of the feed was weighed and the internal standard D_3-flubendazole (30 μL of 100 μg/mL) was added. After the addition of 500 μL of K_2CO_3 4 M in water and 5 mL of ethyl acetate, the samples were vortexed for 1 min and placed on a shaker for 30 min. Samples were centrifuged at 4,700 × g for

5 min at 4°C, the supernatant was transferred into a 15-mL tube, reextraction was performed with 5 mL of ethyl acetate, and both supernatants were pooled. One milliliter was evaporated to dryness at 50°C under nitrogen, 5 mL of hexane was added, and samples were vortexed. One milliliter of an ethanol-HCl 0.2 M solution was added, samples were vortexed for 1 min, which was followed by shaking for 30 min, and centrifugation for 5 min at 2,600 × g at 4°C. The hexane was removed and samples were evaporated to dryness at 50°C under nitrogen. After redissolving with 4 mL of HPLC·H_2O + 0.1% formic acid, the samples were diluted by adding 100 µL of the extract to a sample vial containing 900 µL of HPLC·H_2O + 0.1% formic acid. For the analysis of tylosin A, 20 g of feed was weighed and 1 mL of the internal standard spiramycine (50 µg/mL) was added. The sample was allowed to stand for 10 min, 100 mL of methanol was added, the tube was vortexed, and subsequently placed on a horizontal shaker for 20 min. Six milliliters of the supernatant was transferred in a tube, centrifuged for 10 min at 3,040 × g at 4°C, and 200 µL of the supernatant was transferred in a vial, containing 800 µL of HPLC·H_2O.

Sample Preparation for the Egg Matrices

For flubendazole analysis, 3 g of homogenized egg matrices were weighed and internal standard (30 µL of 1 µg/mL) was added. A nearly identical procedure as described for the feed sample preparation was used. The supernatants were pooled and the complete sample, instead of 1 mL, was evaporated to dryness. After the last evaporation to dryness, extracts were redissolved with 4 mL of HPLC·H_2O + 0.1% formic acid and placed in an HPLC vial. For tylosin analysis, 1 g each of whole egg, egg white, and egg yolk was weighed. The internal standard spiramycine (100 µL of 10 µg/mL) was added, samples were vortexed, and allowed to stand for 5 min. Five milliliters of methanol was added; samples were vortexed and placed on a rotary shaker for 20 min. After centrifugation (4°C, 10 min, 3,040 × g), the supernatant was transferred into a new tube, and then 500 µL of a 10% acetic acid solution in HPLC water was added and samples were vortexed. Ten milliliters of hexane was added, samples were vortexed, placed on a rotary shaker for 10 min, and then centrifuged for 10 min at 3,040 × g at 4°C. The hexane was removed and the extract was transferred into a new tube, evaporated to dryness, and redissolved in 250 µL of HPLC·H_2O. The samples were mixed by vortex and transferred into a cup, which was ultra-centrifuged for 10 min at 12,000 × g at 4°C. The supernatant was transferred into an HPLC vial.

Liquid Chromatography Tandem Mass Spectrometry Analysis

The analysis of flubendazole was performed on a Waters Alliance 2695 Separation Module combined with a Waters Micromass Quattro Ultima MS instrument. A Symmetry column (C18 5 µm, 150 × 2.1 mm) was used. Analysis was performed with a gradient of 0.1% formic acid in HPLC·H_2O and 0.1% formic acid in acetonitrile. The tylosin A analysis was performed on a similar LC-MS system as for flubendazole analysis. Separation was performed on a PLRP-S polymer column (5 µm, 150 × 2.1 mm). A gradient mobile phase, HPLC·H_2O + 0.01 M ammonium acetate (pH 3.5) and acetonitrile, was used. The analysis of both molecules was performed in MRM mode and electrospray positive ionization mode. For flubendazole, the parent molecule and the 2 major metabolites, that is, the reduced and the hydrolyzed forms, were detected (flubendazole: m/z: 314.4 > 123.4, 314.4 > 282.3; H-flubendazole: m/z: 256.4 > 123.4, 256.4 > 95.4; R-flubendazole: m/z: 316.2 > 284.2, 316.2 > 97.2). Tylosin A was the marker residue of tylosin (tylosin A: m/z: 916.4 > 174.1). In this study, the limit of quantification (**LOQ**) of the analytical method for feed was 250 µg/kg and 1.25 mg/kg for flubendazole and tylosin, respectively. For egg matrices, the method LOQ was 1 and 2.5 µg/kg for flubendazole and tylosin, respectively.

Pharmacokinetic and Statistical Analysis

Because the treatments of this study were not repeated, no statistically obvious conclusions about the influence of dosing regimen on weight and number of eggs can be made. Due to the limited data set, no statistical analysis (correlation factors) could be performed on the residue concentrations and physicochemical and pharmacokinetic parameters. Pharmacokinetic (PK) functions for Excel (Microsoft Corp., Redmond, WA) were used to calculate the terminal elimination half-life in egg matrices, and calculation was based on the terminal slope of the tissue concentration-time curve after withdrawal of the experimental feed (Usansky et al., 2011).

RESULTS

Zootechnical Parameters

Zootechnical parameters, such as egg weight and number of eggs, were noted daily. This study occurred only once for each treatment group, therefore, no statistical analysis on the available data can be performed. Nevertheless, no large differences in mean egg weight and mean laying percentage were observed between the different concentration groups for the same period and between the different periods for the same group. During this study, performance in general was high for both molecules, which is demonstrated by the mean laying rate and relatively favorable feed conversion ratios. A mean laying percentage of 92.43 ± 1.84% and 89.33 ± 0.56% and a mean feed conversion of 1.97 ± 0.05 g and 1.94 ± 0.06 g were calculated for the flubendazole and tylosin experiment, respectively.
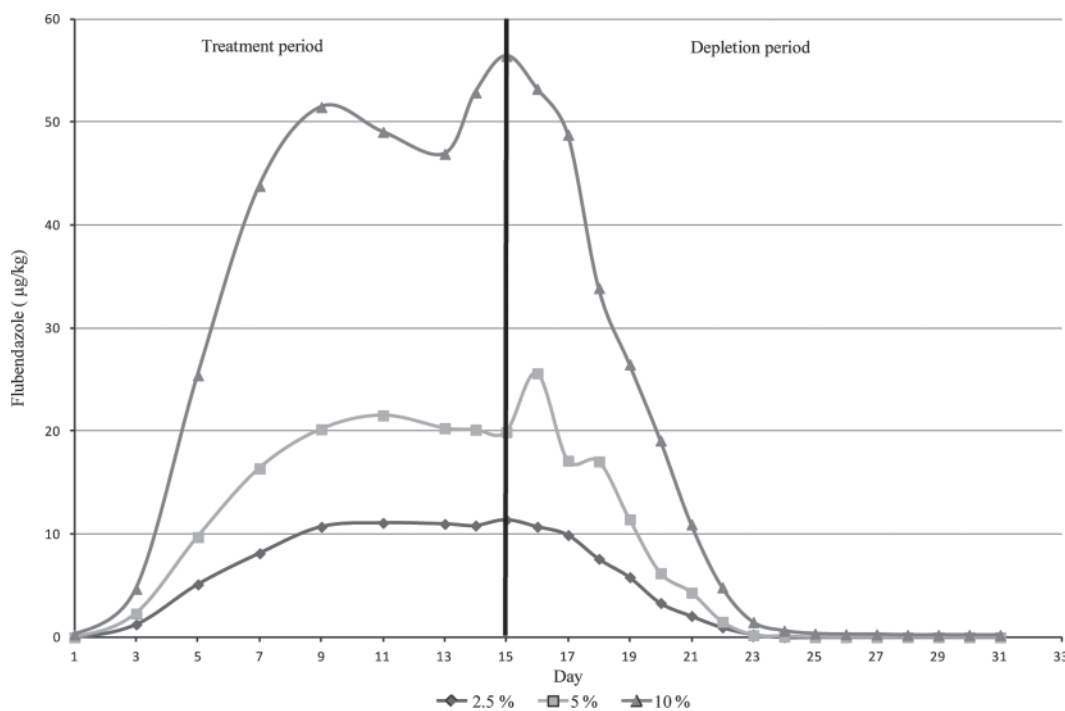
Figure 1. Residue concentrations (μg/kg) in whole egg of flubendazole for 3 concentration groups (2.5, 5, and 10% concentration groups) during the treatment period (d 1–14 included) and the depletion period (d 15–31 included).

Experimental Feed

The maximum allowed doses for flubendazole and tylosin in feed are 30 mg/kg and 100 mg/kg respectively, thus values of 750, 1,500, and 3,000 μg/kg for flubendazole and 2.3, 4.5, and 9.09 mg/kg for tylosin A were expected for the 2.5, 5, and 10% concentration group, respectively. Concentrations of 790 ± 54 (105%), 1,504 ± 160 (100%), 2,707 ± 126 (90%) μg/kg and 2.04 ± 0.83 (90%), 4.58 ± 2.17 (100%), 9.42 ± 2.63 (103%) mg/kg were reached for flubendazole and tylosin A, respectively.

Residue Concentrations in Egg Matrices

For tylosin, no residue concentrations were measured above LOQ for all concentration groups in all egg matrices. Because of this, no transfer factors and no elimination half-lives could be determined.

Flubendazole and its metabolites reached a plateau phase rather slowly and also showed a slow depletion. The residue concentrations of flubendazole and both metabolites in whole egg are presented in Figures 1 and 2, respectively. For flubendazole, the plateau phase was reached at d 9 of the treatment period for all concentration groups. Residue concentrations of flubendazole below LOQ were observed at d 8, 9, and 10 of the depletion period for the 2.5, 5, and 10% concentration groups, respectively. For both metabolites, the plateau phase was reached at d 7 for the 10% concentration group and at d 9 for the 2.5 and 5% concentration groups. The residue concentrations of the metabolites dropped below LOQ at d 6 of the depletion period for

the 2.5 and 5% concentration groups and at d 9 for the 10% concentration group. The 3 groups showed linearity in concentrations reached for the parent molecule as well as for the metabolites in whole eggs. The highest concentrations were measured for the parent molecule and residue concentrations of the hydrolyzed and the reduced metabolites were in the same range, that is, 20 to 25% of the parent molecule.

Transfer factors, calculated by dividing the measured concentration in the 10% group on d 13 of the treatment period by the reached feed concentration is determined for the parent molecule as well as for both metabolites for all matrices. Residue concentrations are expected to reach a plateau level on d 13 of the treatment period for the different egg matrices. Transfer factors in whole egg of 0.017, 0.0037, and 0.0048 are calculated for the parent molecule, the hydrolyzed metabolite, and the reduced metabolite, respectively. Elimination half-lives were calculated for the parent molecule and both metabolites. For the parent molecule, elimination half-lives of 2.06, 1.58, and 1.44 d were calculated for the 2.5, 5, and 10% groups, respectively. The 2.5, 5, and 10% group showed elimination half-lives of 3.4, 1.49, and 2.07 d for the hydrolyzed metabolite and 2.77, 1.88, and 2.12 d for the reduced metabolite, respectively.

The highest concentrations of flubendazole and its metabolites were detected in egg yolk. The residue concentrations of flubendazole and both metabolites for the 10% concentration group in the different egg matrices are presented in Figures 3 and 4, respectively. For the parent molecule and the metabolites, residue concentrations were highest in egg yolk followed by the whole egg, with the lowest concentrations measured in the egg
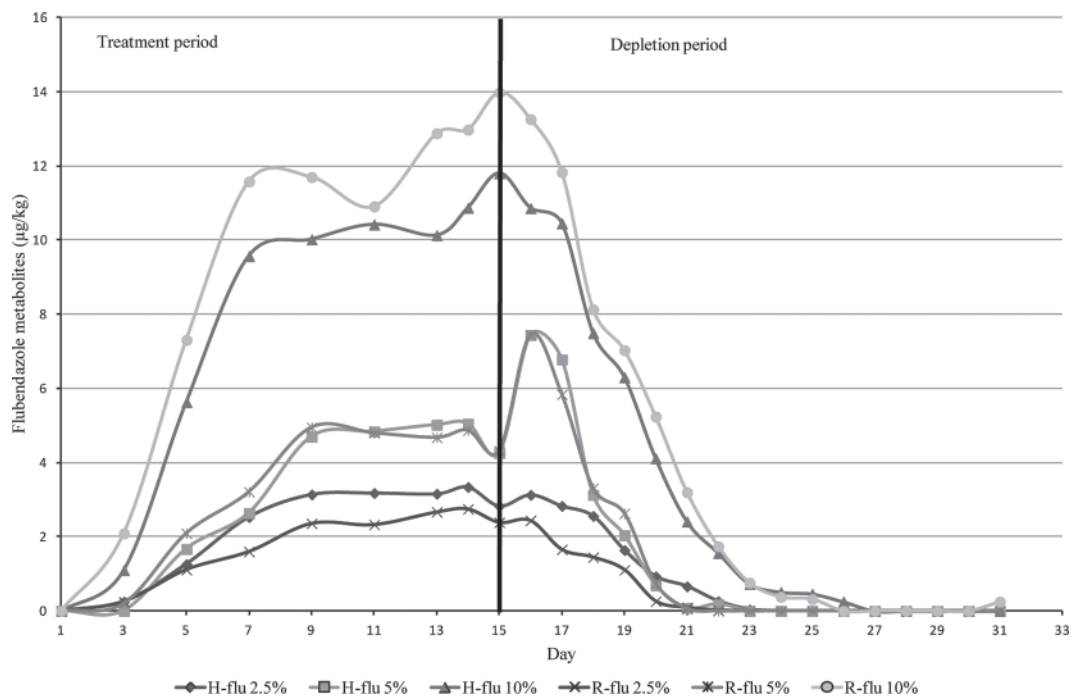
Figure 2. Residue concentrations (µg/kg) in whole egg of H-flubendazole (H-flu) and R-flubendazole (R-flu) for 3 concentration groups (2.5, 5, and 10% concentration groups) during the treatment period (d 1–14 included) and the depletion period (d 15–31 included).

white. The residue concentrations of metabolites in egg white of the 2.5 and 5% concentration groups were below LOQ, whereas both metabolites in the 10% concentration group and the parent molecule in the 2.5% concentration group were around LOQ. Egg white transfer factors of 0.0018, 0.00037, and 0.00079 and egg yolk transfer factors of 0.047, 0.012, and 0.011 were determined for flubendazole, the hydrolyzed metabolite, and the reduced metabolite, respectively. On d 14 of the treatment period, the ratios of egg yolk/egg white were 25, 30, and 14 for the parent molecule, the hydrolyzed, and the reduced metabolite, respectively. Flubendazole residue concentrations were higher compared with the residues of the metabolites in both matrices while residues of the metabolites were in the same range.

Physicochemical and Pharmacokinetic Parameters

In Table 1, physicochemical parameters, such as molecular weight, lipophilicity parameters, and pKa, the pharmacokinetic parameters plasma protein binding and volume of distribution (Lewicki 2006) and the measured concentrations in the different egg matrices for the parent molecule are listed. Different databases have been used that resulted in various (predicted as well as experimental) values for the physicochemical parameters partition coefficient (log P) and pKa (ALOGPS, 2005; Epi Suite; PhysProp Database; SciFinder Scolar).

DISCUSSION

Flubendazole was the major component in all egg matrices. The plateau phase was reached within 9 d,

and values dropped below LOQ at 8 to 10 d once the experimental feed was no longer provided. These findings have been confirmed by Kan et al. (1998); however, a transfer ratio of 0.0038 (transfer factor in our study: 0.017) was mentioned in a study in which the feed was only provided for 7 d at a concentration of 60 mg of flubendazole/kg of feed (EMEA, 2006). Although large differences were observed between residue concentrations of both metabolites in different egg matrices (egg yolk > whole egg > egg white), no large difference in residue concentration was observed between the hydrolyzed and reduced metabolite in each of the egg matrices. This was not confirmed by Kan et al. (1998), who found higher concentrations of the hydrolyzed metabolite compared with the reduced metabolite. Flubendazole is rapidly absorbed in laying hens and there is no evidence of bioaccumulation, but a keto reduction in the liver occurs (EMEA, 2006). Despite a strong metabolization in the liver taking place, no differences in disappearance rate were observed between flubendazole and its metabolites (Kan et al., 1998), and the percentage of residues present as flubendazole remained constant (EMEA, 2006) after withdrawal of the feed. No tylosin A concentrations above LOQ could be detected in the whole egg for the 3 concentration groups. Other researchers have also detected very low transfer ratios from the feed to the whole egg (EMEA, 2000; Furusawa, 2001; Hamscher et al., 2006; Lewicki, 2006). Tylosin reached constant values 4 d after the start of experimental feeding (Furusawa, 2001), but variations in residue concentrations in individual hen's eggs have been noticed (Hamscher et al., 2006).

Flubendazole as well as its metabolites are mostly present in the egg yolk, which Kan et al. (1998) also
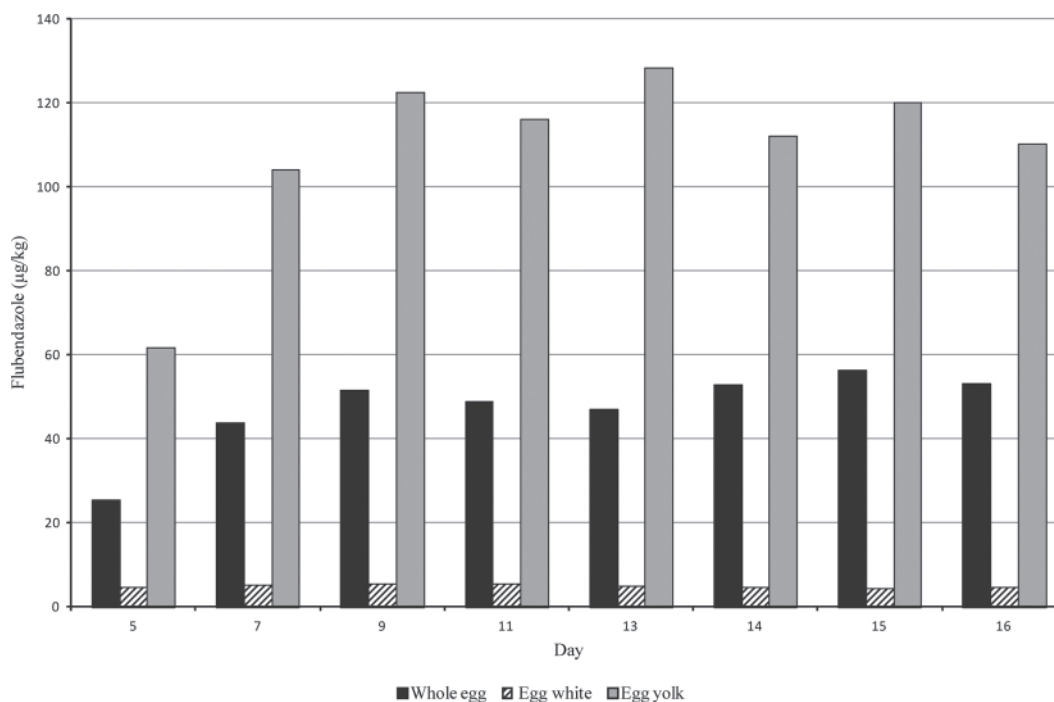
Figure 3. Residue concentrations (μg/kg) in whole egg, egg white, and egg yolk of flubendazole for the 10% concentration group during the treatment period (d 5–14 included) and the depletion period (d 15–16).

observed. No tylosin A residues above LOQ were measured in egg yolk and in egg white, therefore no conclusions about difference in distribution can be made. Other researchers also determined tylosin residue concentrations in whole egg, egg white, and egg yolk (Yoshida et al., 1973; Lewicki, 2006). When laying hens received feed containing tylosin at a dose of 8,000 g/ton for 7 d, mean residue concentrations in egg white and egg yolk were 2,818 and 1,820 μg/kg on d 3 of the treatment period, 5,160 and 5,150 μg/kg on d 7 of the treatment period, and 3,930 and 4,840 μg/kg on d 1 of the depletion period. Residue concentrations were higher in egg yolk than in egg white in 20% of the eggs on d 3 of the treatment period and in 60% of the eggs
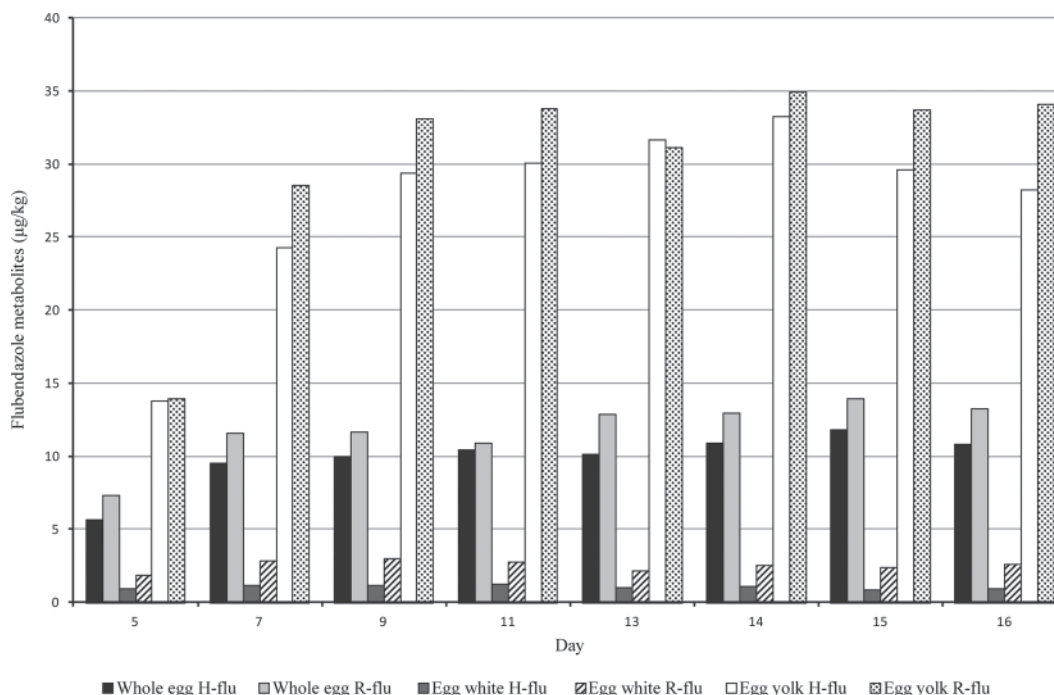

Figure 4. Residue concentrations (μg/kg) in whole egg, egg white, and egg yolk of H-flubendazole (H-flu) and R-flubendazole (R-flu) for the 10% concentration group during the treatment period (d 5–14 included) and the depletion period (d 15–16).

Table 1. Various physicochemical and pharmacokinetic parameters and residue concentrations of flubendazole and tylosin

Item	Flubendazole	Tylosin	Reference
Molecular weight	313.28	916.1	SciFinder Scholar, 2011
Molecular weight	313.29	916.12	PhysProp Database, 2011
pKa (most acidic)	10.66	13.06	SciFinder Scholar, 2011
pKa (most basic)	4.45	7.39	SciFinder Scholar, 2011
pKa	—	7.73	PhysProp Database, 2011
Log D (pH = 7)	3.08	0.15	SciFinder Scholar, 2011
Log P	3.084	0.628	SciFinder Scholar, 2011
	2.91	1.63	PhysProp Database, 2011
	2.91	1.05	EPI Suite, 2011
Plasma protein binding, %	—	30%	Lewicki, 2006
Volume of distribution, L/kg	—	0.69	Lewicki, 2006
Concentration (μg/kg) in whole egg at d 13 of treatment period	46.91	<LOQ[1]	
Concentration (μg/kg) in egg white at d 13 of treatment period	4.91	<LOQ	
Concentration (μg/kg) in egg yolk at d 13 of treatment period	128.48	<LOQ	

[1]LOQ = limit of quantification.

on d 7 of the treatment period and on d 1 of the depletion period. Residue concentrations in decreasing order were found in egg yolk, whole egg, and egg white starting on d 3 of the treatment period, when laying hens received tylosin via their drinking water for 5 d (Yoshida et al., 1973; Lewicki, 2006). Several authors mentioned differences in distribution of veterinary drugs between egg white and egg yolk, and various hypotheses have been suggested (Kan, 2003; Lewicki, 2006). According to Furusawa, drugs that are lipid soluble are found in a higher concentration in egg yolk than in albumen (Furusawa, 2001). A log P value >1 defines a drug as lipophilic (Grabowski and Jaroszewski, 2009). Due to the variation on values for log P provided by different databases, it is difficult to find correlations between the physicochemical parameters of the molecule and the preference of a molecule for egg white or egg yolk. Besides the physicochemical and pharmacokinetic parameters, metabolization and elimination processes may also influence drug residue concentrations. Flubendazole is highly metabolized in the liver, and primary metabolism of tylosin also occurs in the liver (EMEA, 2006; Lewicki, 2006). Yolk components, very low-density lipoproteins, vitellogenin, vitamin-binding proteins, and immunoglubulins are formed in the liver, transported via the blood, and are actively and specifically taken up by the growing oocytes (Nimpf and Schneider, 1991; Kan, 2003). De novo lipogenesis does not occur in the ovary, therefore, triglyceride storage depends on the availability of plasma lipid substrate. The lipid substrate in the plasma originates from either the diet or lipogenesis in the liver. Commercial poultry diets are usually lipid-poor (less than 10%); therefore, the liver plays an important role in lipid provision. Hepatic lipogenesis is dramatically enhanced in laying hens due to the demand for vitellogenesis, triglycerides are the main products of de novo lipogenesis. However, the liver is also the major site of cholesterol and phospholipids synthesis, which are the main components of lipoproteins, in combination with proteins. Lipoproteins are generally classified as either very low-density or high-density lipoproteins. Due to the very limited catabolisation of very low-density lipoproteins in plasma

of laying hens, lipids are transported to the oocytes, where very low-density lipoproteins are endocytosed, rather than to other tissues (Hermier, 1997). Because flubendazole is highly metabolized in the liver, and the liver is the main source of very low-density lipoproteins, we hypothesize that this might also contribute to the deposition of the drug in the egg yolk.

Some veterinary drugs used in laying hens may result in residues being retained in the eggs. This study demonstrates that the presence of flubendazole in feed of laying hens caused by cross contamination may lead to detectable levels in eggs, although no MRL is exceeded. Transfer of tylosin from the feed to the egg matrices was very low. The MRL are set for whole egg, although highest flubendazole as well as tylosin concentrations were measured in the egg yolk. Sampling of the different egg matrices is recommended to ensure food safety. Flubendazole and tylosin A are retained as marker residues and MRL are set for the whole egg. Given that the parent molecule flubendazole was present in higher concentrations compared with the metabolites, indeed flubendazole must be assigned as the marker residue.

ACKNOWLEDGMENTS

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