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**Residues of sulfadiazine and doxycycline in broiler liver and muscle tissues due to cross-contamination in the feed**

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Abstract:	Veterinary drugs, such as antimicrobial compounds are widely used in poultry and may lead to the presence of residues in matrices of animal origin such as muscle and liver tissue. In this study, broilers received experimental feed, containing sulfadiazine or doxycycline at cross contamination levels of 2.5%, 5% and 10 % of the therapeutic dose in feed. Breast- and thigh muscle and liver samples were collected during treatment and depletion period and analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Concentrations reached a plateau phase 3 to 5 days after starting the experimental feed. A rapid depletion of residues was noted after withdrawal of the experimental feed. No significant differences in measured concentrations were observed between the different muscle types. The residue concentrations for some experimental groups, the 10 % group of sulfadiazine and the 5% and 10 %

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	group of doxycycline, however exceeded the corresponding Maximum Residue Limits (MRLs).

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## Residues of sulfadiazine and doxycycline in broiler liver and muscle tissues due to cross-contamination of the feed

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### Abstract

Veterinary drugs, such as antimicrobial compounds are widely used in poultry and may lead to the presence of residues in matrices of animal origin such as muscle and liver tissue. In this study, broilers received experimental feed, containing sulfadiazine or doxycycline at cross-contamination levels of 2.5%, 5% and 10 % of the therapeutic dose in feed. Breast- and thigh muscle and liver samples were collected during treatment and depletion period and analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Concentrations reached a plateau phase 3 to 5 days after starting the experimental feed. A rapid depletion of residues was noted after withdrawal of the experimental feed. No significant differences in measured concentrations were observed between the different muscle types. The residue concentrations for some experimental groups, the 10% group of sulfadiazine and the 5% and 10% group of doxycycline, however exceeded the corresponding Maximum Residue Limits (MRLs).

**Keywords:** Residue; sulfadiazine; doxycycline; muscle; liver; feed; cross-contamination; depletion

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## 31 Introduction

32 The veterinary drugs used therapeutically in poultry industry are generally given via mass  
33 application in feed or drinking water (Kan and Petz 2000). Antibiotics are used to raise  
34 production efficiency , as they enhance growth and feed efficiency and reduce disease  
35 (Donoghue 2003). Antibiotics have been used as growth promoters in the member states of  
36 the EU but concerns about development of antibacterial resistance led to the withdrawal of  
37 these agents as growth promoters in the EU since January 1, 2006 (Castanon 2007). Some  
38 antimicrobial drugs, such as sulfonamides are used to treat coccidiosis (Roudaut and Garnier  
39 2002; Sentepe and Eraslan 2010). In veterinary medicine the sulfonamides are effective  
40 chemotherapeutics for bacterial or protozoal diseases (EMA 1995; BCFI 2010). Doxycycline  
41 belongs to the group of tetracycline antibiotics, which have a broad spectrum activity (EMA  
42 1996). These drugs are widely used as mass medication in both pig and poultry industry.  
43 Concerning feed medication, in Belgium, sulfadiazine in combination with trimethoprim is  
44 available as pre-mix for pigs and non-laying hens and as oral powder to be mixed in the feed  
45 of pigs. Doxycycline is registered as a premix only for pigs and also an oral powder to be  
46 mixed in the feed of pigs is available (BCFI 2010). These drugs work systemically meaning  
47 that they must cross the intestinal wall and distribute in the body to exert their function (Kan  
48 and Petz 2000). After absorption from the gastrointestinal tract, the drug reaches the blood  
49 stream and is distributed throughout the whole body. This distribution can be quantified by  
50 some pharmacokinetic characteristics such as volume of distribution, which is largely  
51 determined by the physicochemical parameters of the compounds (Kan and Petz 2000),  
52 plasma and tissue protein binding and animal physiological status (Toutain and Lees 2004).

53 Residues exceeding established safety tolerances may be the result of misuse of  
54 antibiotics, due to misreading of the product label or not respecting the withdrawal time or  
55 the accidental administration of feed contaminated with pharmacologically active residues  
56 (Reyes-Herrera et al. 2005; Segato et al. 2011). According to Regulation 178/2002, a feed or  
57 feeding stuff is any substance or product, including additives, whether processed, partially  
58 processes or unprocessed, intended to be used for oral feeding to animals. In Directive  
59 2001/82/EC, a medicated feeding stuff is described as any mixture of a veterinary medicinal  
60 product or products and feed or feeds that is ready prepared for marketing and intended to  
61 be fed to animals without further processing, because of its curative, preventive or other

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3 62 properties. Medicated feeding stuffs are prepared in multi-product plants following good  
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5 63 manufacturing practice guidelines (Segato et al. 2011). Carry-over, transfer from one  
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7 64 production batch to the following batch may result in cross-contamination and may occur  
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9 65 during feed processing for many reasons (Borras et al. 2011). In the EFSA reports, cross-  
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11 66 contamination is defined as: “contamination of feeds that are produced after the production  
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13 67 of a mixed feed, containing additives with residual amounts of the previous feed batch”.  
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15 68 Cross-contamination may be product or establishment related. Some feed additives and  
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17 69 premixes properties; adhesive strength-adhesion to walls, particle size and density (carrier,  
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19 70 substance) and electrostatic properties influence cross-contamination behavior and affect  
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21 71 how cross-contamination occur. The technological equipment in the feed mill; the design of  
22  
23 72 dosage and grinding and mixing equipment can influence the level of cross-contamination  
24  
25 73 (EFSA 2007; Borras et al. 2011). Under practical conditions, cross-contamination of residues  
26  
27 74 is unavoidable, even when preventive measures are taken (EFSA 2007). A totally risk/residue  
28  
29 75 free food production system does not exist. Consequently, active surveillance and  
30  
31 76 compliance programs to ensure the proper use of antibiotics and the safety of the food  
32  
33 77 supply are needed (Sasanya et al. 2005). Many governments have established antibiotic  
34  
35 78 residue tolerances in edible animal tissues and have determined the target tissues for  
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37 79 residue monitoring (Reyes-Herrera et al. 2005). In the European Union, these Maximum  
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39 80 Residue Limits (MRLs) for doxycycline for pigs and poultry are set at 100  $\mu\text{g kg}^{-1}$  (muscle),  
40  
41 81 300  $\mu\text{g kg}^{-1}$  (skin and fat, liver) and 600  $\mu\text{g kg}^{-1}$  (kidneys) (EMEA 1996; BCFI 2010). For all  
42  
43 82 sulphonamides, the MRL is 100  $\mu\text{g kg}^{-1}$  in muscle, fat, liver and kidneys for all food-producing  
44  
45 83 animals (AFSCA 2004; BCFI 2010). Taking into account the unavoidable cross-contamination  
46  
47 84 in practical field conditions, the aims of this study were to investigate the transfer ratios of  
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49 85 two frequently used drugs from the feed to poultry edible tissues when provided at cross-  
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51 86 contamination levels and to evaluate this transfer in relation to current MRL's.

## 87 **Material and methods**

### 88 ***Premix and oral powders, reagents and standards***

89 The premix containing sulfadiazine and thrimetoprim was Tucoprim<sup>®</sup> powder, kindly  
90 provided by Pfizer (Brussels, Belgium). Doxycycline oral powder to be mixed in the feed was  
91 Doxycycline 75 % Kela<sup>®</sup>, kindly provided by Kela Veterinaria (Sint-Niklaas, Belgium).  
92 Analytical standards of sulfadiazine and doxycycline hyclate were from Sigma (Bornem,

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3 93 Belgium) and Acros (Geel, Belgium), respectively. The internal standards, namely  
4 94 sulfachloropyridazine and demethylchlortetracycline were purchased from Sigma.  
5 95 Acetonitrile, distilled water and methanol came from Biosolve (Valkenswaard, The  
6 96 Netherlands). Water was HPLC grade and generated by a Q Gard 2 system (Millipore,  
7 97 Billerica, MA, USA). The anhydrous sodium sulphate came from Merck (Darmstadt,  
8 98 Germany). Filters for filtration of the sulfadiazine extract, i.e. 0.22 µm Millex® GV were from  
9 99 Millipore (Billerica, MA, USA). The solid-phase extraction (SPE) columns used for the clean-  
10 100 up of doxycycline containing samples were Oasis® columns (HLB SPE column 60 mg/3 ml,  
11 101 Waters, Milford, MA, USA). The Robot Coupe 2, used to mince muscle and liver samples,  
12 102 came from C-Tech Systems (Lille, Belgium).

### 103 ***Preparation of the experimental feed and animal experiments***

104 The feed preparation and the animal experiments were carried out at ILVO's Animals  
105 Sciences Unit with approval from the ILVO ethical committee (EC no. 2008/89, 2009/108).

106 The experimental feed was mixed with sulfadiazine or doxycycline at cross-  
107 contamination levels of 10 %, 5 % and 2.5 % of the maximum allowed dose of 250 mg kg<sup>-1</sup>  
108 feed for both drugs. The premix resp. oral powder was first mixed with a small amount of  
109 blank feed in the Varimixer Bear (Dehaeck construct, Ghent, Belgium), which was then  
110 added to the remaining blank feed in the feed mixer constructed by Silobouw (Zulte,  
111 Belgium). The final experimental feed was mixed at 35 rpm during 30 minutes with a switch  
112 of direction every five minutes. Before and between the preparations of each experimental  
113 feed, rinsing batches were carried out. Ten samples were taken from the moving stream of  
114 experimental feed and from the top (n=3), the middle (n=4) and the bottom (n=3) of each  
115 experimental feed to determine the concentration achieved and two samples of each rinsing  
116 batch were analyzed to exclude cross-contamination. Because of the large amount needed,  
117 the preparation of each experimental feed was performed in two phases.

118 A growth trial was set-up with 575 one-day-old broilers, male Ross 308, which were  
119 housed per concentration in a concrete floor pen under conventional conditions of  
120 ventilation, temperature and lighting (22 hours light/day). The animals were randomly  
121 assigned to three treatments, distributed among three pens and separated from each other  
122 by a narrow walkway. Each study group counted 188 chickens, of which 102 were

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3 123 euthanized. A small group of 11 broilers was used as control group. Each study consisted of a  
4 124 standard 2-phase feeding including a starter and grower diet. After an adaptation period of  
5 125 12 days, experimental feed was provided during 14 days, followed by a depletion period of  
6 126 17 days, during which the animals received blank feed. The animals were given free access to  
7 127 water and feed; they were vaccinated for Infectious Bronchitis (IB), New Castle Disease  
8 128 (NCD) and were treated for coccidiosis. The diets were supplemented with an anticoccidial  
9 129 (salinomycine at 60 ppm for 1-35 days) and a NSP enzyme (Ronozyme WX at 50 FXU/kg feed)  
10 130 but without any growth promoting antibiotic. The animals and housing facilities were  
11 131 inspected daily for general and individual health, sufficient feed and water supply,  
12 132 temperature and ventilation as well as for dead animals and unexpected events. The  
13 133 zootechnical parameter, chicken weight, was noted at day 10 of pre-treatment period, days  
14 134 1, 3, 5, 7, 9, 11, 13 of treatment period and days 1, 3, 5, 7, 9, 11, 13, 15, 17 of post treatment  
15 135 period. From the age of 10 days onwards (day 10 of pre-treatment period), six animals per  
16 136 concentration group were slaughtered by cervical dislocation every 2 days, chicken weight  
17 137 was noted, breast feathers were removed and the complete breast muscles, the upper thigh  
18 138 muscles and the liver (without the gall-bladder) were sampled. All samples were minced and  
19 139 grounded with a Robot Coupe 2 and stored at -18 °C until analysis. A pooled sample was  
20 140 prepared by mixing 5 to 10 g of every specific tissue from each of the 6 chickens.

21 141 Analysis of the breast muscle was performed every 2-days starting from day 10 of the  
22 142 pre-treatment period. Samples from the upper thigh muscles and the liver were only  
23 143 analyzed for the 10% carry-over concentration and only during steady state.

#### 24 144 ***Extraction and clean-up of feed samples***

25 145 For the analysis of sulfadiazine, 5 g of feed was weighed, 100 µL of the internal standard  
26 146 sulfachloropyridazine (1 mg mL<sup>-1</sup>) was added and the sample was allowed to stand for 10  
27 147 minutes. After addition of 25 mL of methanol, the tube was vortex mixed and afterwards  
28 148 placed on the horizontal shaker for 30 minutes. After centrifugation during 10 minutes at  
29 149 3000 rpm, 5 mL of the supernatant was transferred into a tube and evaporated under  
30 150 nitrogen to dryness in a water bath at 45 ° C. After redissolving the residues in 10 ml  
31 151 acetonitrile:water (ACN: H<sub>2</sub>O, 50:50), a dilution of 1:15 was performed in ACN:H<sub>2</sub>O followed  
32 152 by filtration using a 0.22 µm Millex®Gv. For the analysis of doxycycline, 5 g of feed was



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3 153 weighed and 1 ml of the internal standard demethylchlortetracycline ( $20 \mu\text{g mL}^{-1}$ ) was  
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5 154 added. After the addition of 25 mL of methanol, the samples were placed on a rotary shaker  
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7 155 for 20 minutes and centrifuged for 10 min at 4000 rpm. Two hundred  $\mu\text{L}$  of the supernatant  
8  
9 156 was transferred in a vial, 800  $\mu\text{L}$  of HPLC water was added and the vial was vortex mixed.

#### 10 11 157 ***Extraction and clean-up of broiler samples***

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13 158 For sulfadiazine analysis in muscle and liver, we used the method described by Mortier et al.  
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15 159 (2005). Two grams of minced muscle or liver were weighed and the internal standard ( $20 \mu\text{L}$   
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17 160 of  $10 \mu\text{g mL}^{-1}$ ) was added. The samples were vortex mixed, were allowed to stand for 10 min  
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19 161 and 6 g (muscle) or 8 g (liver) of anhydrous sodium sulphate was added. The tissue was  
20  
21 162 carefully mixed with a spatula until a powdery mixture was obtained and 10 mL (muscle) or  
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23 163 20 mL (liver) of acetonitrile was added. After vortex mixing, the tubes were placed on a  
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25 164 horizontal shaker for 30 min. The tubes were centrifuged (15 min, 4000 rpm), 5 mL (muscle)  
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27 165 or 10 mL (liver) of the supernatant was transferred into a tube and was evaporated to  
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29 166 dryness under nitrogen in a water bath of  $45^\circ\text{C}$ . Then, the sample was redissolved in 1 mL of  
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31 167 an acetonitrile/water mixture (50:50,v:v) containing 0.1 % formic acid, vortex mixed,  
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33 168 sonicated for 5 min and filtered through a  $0.22\mu\text{m}$  filter into a HPLC vial.

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35 169 For doxycycline analysis in tissues, 2 g of minced tissue were weighed and extracted  
36  
37 170 as described by Cherlet et al (2003). After the addition of the internal standard  
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39 171 demethylchlortetracycline ( $50 \mu\text{L}$  of  $10 \mu\text{g mL}^{-1}$ ), the samples were vortex mixed and were  
40  
41 172 allowed to stand for 5 min. Ten mL of a 0.1 M sodium succinate buffer was added, the  
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43 173 samples were placed on a rotary shaker for 20 minutes and centrifuged at 4000 rpm for 10  
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45 174 min. The supernatant was added to a centrifuge tube containing 1 mL of a 20 %  
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47 175 trichloroacetic acid, vortex mixed and centrifuged at 4000 rpm for 10 min. The supernatant  
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49 176 was filtered through a Whatman® filter paper and was ready for a further solid-phase clean-  
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51 177 up step. In the solid-phase clean-up, the Oasis® HLB (hydrophilic lipophilic balance) column  
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53 178 was preconditioned consecutively with 3 mL methanol, 3 mL of a 1N HCl solution and 3 mL  
54  
55 179 of water and the extract was allowed to pass slowly through the HLB column. The HLB  
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57 180 column was washed with 1 mL of water and dried. The analytes were eluted with 3 mL of  
58  
59 181 methanol and the eluate was evaporated to dryness at  $40^\circ\text{C}$  under nitrogen. After  
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182 redissolution with 250  $\mu\text{L}$  of a 0.5 % formic acid solution and vortex mixing, the sample was  
183 transferred to an autosampler vial.

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3 184 **LC-MS/MS analysis**  
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5 185 The sulfadiazine analysis was performed on a Waters Alliance 2695 Separation Module  
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7 186 combined with a Waters Micromass Quattro MS instrument. An X-terra® column (C<sub>18</sub> 5 µm,  
8  
9 187 150 x 2.1 mm) was used and the analysis was performed with a gradient of 0.1 % formic acid  
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11 188 (FA) in H<sub>2</sub>O (solvent A) and 0.1 % FA in ACN (solvent B). The analysis of doxycycline was  
12  
13 189 performed on a Waters Alliance 2695 Separation Module combined with a Waters  
14  
15 190 Micromass Quattro Ultima MS instrument. Separation was performed on a PLRP-S polymer  
16  
17 191 column (5 µm, 150 x 2.1 mm). An isocratic mobile phase consisting of 70 % solvent A (HPLC  
18  
19 192 H<sub>2</sub>O+0.5% formic acid and 0.001 M oxalic acid; 0.5 % tetrahydrofurane) and 30 % of solvent  
20  
21 193 B (ACN) was used. The analysis of sulfadiazine and doxycycline was performed in MRM mode  
22  
23 194 and electrospray positive ion mode (sulfadiazine: *m/z* 250.94 -> 107.95, 250.94 -> 92.18,  
24  
25 195 250.94 -> 156.06; doxycycline: *m/z* 462.45 -> 428.1). In this study, the limit of quantification  
26  
27 196 (LOQ) of the method was 5 mg kg<sup>-1</sup> and 2.5 mg kg<sup>-1</sup> for sulfadiazine and doxycycline  
28  
29 197 respectively for feed. For muscle and liver, the method LOQ was 2 and 10 µg kg<sup>-1</sup> for  
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31 198 sulfadiazine and doxycycline.

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199 **Pharmacokinetic and statistical analysis**

200 These studies were not repeated, therefore, no statistical analysis of the effect of the  
201 experimental feed on the zootechnical parameters was performed. Due to the limited  
202 dataset of the experimental feed, no statistical analysis to determine the homogeneity of the  
203 experimental feeds could be carried out. The terminal elimination half-life and elimination  
204 rate constant in tissues were calculated using pharmacokinetic (PK) functions for Excel and  
205 were based on the terminal slope of the tissue concentration-time curve after withdrawal of  
206 the experimental feed (Usansky et al, Allergan, Irvine, USA). Statistical analysis was  
207 performed with Statistica 9.0 (StatSoft. Inc., Tulsa, OK, USA). Significance level  $\alpha$  was set at  
208 0.05. Statistical analysis was performed on the mean of the residue concentrations of the  
209 individual chickens and the pooled sample for each of the different matrices. The  
210 comparison between measured residue concentrations in thigh muscle and in breast muscle  
211 was performed with a t-test for dependent samples. The pooled sample on day 13 of  
212 treatment period was analysed six times. The mean of these results was compared with the  
213 mean of the individual chickens by an independent t-test.

**214 Results****215 Zootechnical parameters and experimental feed**

216 The effects of experimental feed on the performance parameter, broiler weight, were  
217 studied. The mean broiler weights are presented for sulfadiazine and doxycycline in Figure 1.

218 For both sulfadiazine and doxycycline the maximum authorized dose is 250 mg kg<sup>-1</sup>  
219 (instruction leaflets). Therefore a value of 25, 12.5 and 6.25 mg kg<sup>-1</sup> was calculated for the 10  
220 %, 5 % and 2.5 % carry-over groups, respectively. Mean concentrations ± standard deviation  
221 and recovery for the 10 %, 5 % and 2.5 % groups were 21.2 ± 1.8 (79 %), 12.7 ± 0.9 (93 %)  
222 and 5.5 ± 0.4 (88 %) mg kg<sup>-1</sup> for sulfadiazine and 24.7 ± 4.6 (99 %), 13.2 ± 4.1 (106 %), 5.5 ±  
223 1.2 (88 %) mg kg<sup>-1</sup> for doxycycline.

**224 Residue concentrations in breast muscle, thigh muscle and liver**

225 The residue concentrations for respectively sulfadiazine and doxycycline for all groups in  
226 breast muscle are shown in Figure 2. The plateau phase was reached at day 3 for all dietary  
227 treatments of the doxycycline and the 2.5 and 5 % carry over group of sulfadiazine. A rapid  
228 depletion phase was noted after withdrawal of the experimental feed. The breast muscle  
229 tissue elimination half-life was calculated for both molecules but due to the rather fast  
230 decline below the LOQ, elimination half-life could only be calculated for the 5 and 10 %  
231 group of doxycycline, namely 3.1 and 3.5 days, respectively. The concentrations in the three  
232 tissue matrices for the 10 % group during plateau phase are presented in Figure 3. Small  
233 differences in concentrations were noted between the different types of muscles for  
234 sulfadiazine as well as for doxycycline but these were not statistically significant. Measured  
235 residue concentrations in liver were similar from the concentrations in muscle for  
236 sulfadiazine although higher concentrations were measured in liver compared to muscle for  
237 doxycycline. The ratio liver/breast muscle was 1.2 and 1.8; the ratio liver/thigh muscle was  
238 1.3 and 1.9 for sulfadiazine and doxycycline respectively. Transfer factors,

239 
$$\frac{\text{concentration in the matrix on day 13 of treatment period for the 10 \% group}}{\text{measured concentration in the feed}} \times 100$$

240 are presented for liver, breast muscle and thigh muscle for sulfadiazine and doxycycline.  
241 Transfer factors of breast muscle, thigh muscle and liver were 1.04, 0.95 and 1.37 % for  
242 sulfadiazine and 1.27, 1.62 and 2.90 % for doxycycline.

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3 243 The measured residue concentrations of the tissues from the 6 individual chickens  
4 244 and the six repetitive analyses' of the pooled sample were compared. The calculated means  
5 245 and standard deviations are presented in Table 1. For both molecules no significant  
6 246 difference between the 2 means was noted for all matrices ( $P>0.05$ ).

## 10 247 **Discussion**

11  
12 248 For the experimental feed of sulfadiazine and doxycycline, the target concentrations of 2.5, 5  
13 249 and 10 % of the maximum allowed dose were reached (79 – 106 %) but no statements can  
14 250 be made about the homogeneity of the experimental feeds. The achievement of adequate  
15 251 mixing of an active ingredient in final feed depends on the composition of the final feed, the  
16 252 precision and the size of samples taken for analysis and other factors such as particle size,  
17 253 electrostatic properties, types of mixing machinery and mixing in stages or trituration (EMEA  
18 254 1996). The ideal way to mix a drug is to add it at the beginning of the pre-mix process just  
19 255 prior to pelleting (Daniel 2009). Good sampling requires sufficient samples of adequate size  
20 256 and sampling from a moving stream is better than static sampling (Borras et al. 2011). Since  
21 257 very small volumes of pre-mixes were added to large amounts blank finished feed and  
22 258 because the experimental feed differs in composition from the feed used for target species  
23 259 for which the medicated pre-mix is intended, it is not easy to guarantee a homogeneous  
24 260 feed. For each experimental feed, ten dynamic samples were taken after 30 min of mixing at  
25 261 the top ( $n = 3$ ), the middle ( $n= 4$ ) and the bottom ( $n=3$ ) of the final feed in order to provide  
26 262 as good as possible sampling procedure.

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29 263 For both compounds studied, no significant differences were found between the  
30 264 concentrations in breast and thigh muscle. Most of the sampling occasions, the residue  
31 265 concentrations in breast muscle were slightly higher compared to the concentrations in thigh  
32 266 muscle. For doxycycline, this was confirmed by Atef et al. (2002) while Reyes-Herrera et al.  
33 267 (2005, 2008) found significantly higher concentrations of enrofloxacin in breast muscle  
34 268 compared to thigh muscle but no difference in concentrations between different breast  
35 269 sections (Reyes-Herrera et al, 2005; Reyes-Herrera et al, 2008). Other authors reported  
36 270 differences between residual concentrations in thigh and breast muscle, which may be  
37 271 explained by a different amount of intramuscular fat content. According to Reyes-Herrera,  
38 272 different antibiotics may have different sites of preferential deposition and therefore it may

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3 273 be interesting to determine the edible tissue with the highest concentration if the target  
4 274 tissue is muscle (Reyes-Herrera et al. 2005). Elimination half-lives were calculated for breast  
5 275 muscle. Atef et al. (2002) found an elimination half-life of 1.8 days for breast muscle in  
6 276 contrast to our calculated value of 3.1 to 3.5 days. This difference may be due to the  
7 277 different ways of oral administration and the duration of the administration, a dose of 15  
8 278 mg/kg was provided orally twice daily during 5 successive days. Doxycycline had higher  
9 279 residue concentrations in the liver compared to muscle as reflected by the feed/tissue  
10 280 transfer ratio's, which has also been described by other researchers (Atef et al. 2002; Ismail  
11 281 and El-Kattan 2004). Doxycycline is known to be well absorbed from the gastro-intestinal  
12 282 tract (Yoshimura et al. 1991), to have a high volume of distribution with the highest detected  
13 283 levels in liver and kidney (EMEA 1996) and to have a high tissue binding (Santos et al. 1997).  
14 284 Doxycycline has an absolute oral bioavailability of 73.4 % and may be metabolised up to 40  
15 285 % by the liver and is largely excreted in faeces, via bile (EMEA 1996; Laczay et al. 2001). After  
16 286 oral dosing, a good bioavailability (approximately 80 %) of sulfadiazine is obtained in non-  
17 287 fasted chickens (Baert et al. 2003). Sulphonamides may be metabolized in various tissues,  
18 288 mainly the liver at varying degrees and through varying mechanisms such as acetylation and  
19 289 oxidation and are mainly excreted via the kidneys (Sentepe and Eraslan 2010).

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34 290 In this study, besides individual tissue analysis we also analyzed a pooled sample. We  
35 291 may conclude that pooled tissue samples are a good reference for the mean of the 6  
36 292 individual chickens for the studied matrices. An important remark is the animal dependent  
37 293 variability, noted for each of the different matrices. This needs to be kept in mind while  
38 294 sampling and interpreting the results in case of a cross contamination problem occurs on a  
39 295 farm.

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45 296 For muscle, MRL's have been set at  $100 \mu\text{g kg}^{-1}$  for sulfadiazine as well as for  
46 297 doxycycline (BCFI 2010). The 10 % group of sulfadiazine and the 5 and 10 % group of  
47 298 doxycycline generated concentrations above the MRL, whereas intake of 5 % carry-over of  
48 299 sulfadiazine and the 2.5 % of doxycycline resulted in concentrations around the MRL. Only  
49 300 the 2.5 % group of sulfadiazine generated concentrations that were below the MRL. For  
50 301 liver, MRL's have been set at  $100 \mu\text{g kg}^{-1}$  and  $300 \mu\text{g kg}^{-1}$  for sulphonamides and doxycycline  
51 302 resp. (BCFI 2010). Only liver tissue from the animals receiving the 10% concentration in feed  
52 303 was analyzed and resulted in concentrations exceeding the MRLs. It may be concluded that  
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3 304 cross-contamination may lead to the presence of residual concentrations above MRL.  
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5 305 However, once the “cross-contaminated feed” was no longer administered, we observed a  
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7 306 fast decline in measured concentrations.  
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9 307 The use of cross-contaminated feed may have several consequences for animal as  
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11 308 well as human health. Besides the possible adverse effect on the health of non-target  
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13 309 species and the indirect toxic effect by the promotion of resistant strains of bacteria, the  
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15 310 MRL may also be exceeded in food matrices of animal origin, and consequently food safety  
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17 311 may not be guaranteed (Donoghue et al. 1997; Segato et al. 2011; Vincent et al. 2011). These  
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19 312 experiments indicate that important residual levels of both molecules occur in both muscle  
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21 313 and liver after feeding cross-contamination levels. An unaware farmer, who provides feed  
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23 314 accidentally contaminated with pharmacologically active residues to his animals, may also  
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25 315 face legal repercussions because of exceeding the MRL in matrices of animal origin.  
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27 316 However, the problem of residual concentrations due to the use of cross-contaminated feed  
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29 317 can be resolved within days after switching to blank feed since both molecules showed quick  
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31 318 residue depletion once the experimental feed was no longer administered.

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324 Eeckhout and G. Van Den Bossche of ILVO's Animal Sciences Unit for their help with the  
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### 326 327 **References**

328 AFSCA. 2004. Advies 24-2004: “Actielimieten voor de aanwezigheid van residuen van  
329 bepaalde additieven en bepaalde diergeneesmiddelen in levensmiddelen wanneer er geen  
330 maximumwaarde voor residuen (MRL) is en waarboven maatregelen moeten worden  
331 getroffen ter vrijwaring van de veiligheid van de voedselketen.” (SciCom2004/24). [Internet].  
332 2004. Federaal Agentschap voor de Veiligheid van de Voedselketen; [cited 2004].  
333 [www.afsca.be/home/com-sci/doc/avis04/Advies\\_2004-24.pdf](http://www.afsca.be/home/com-sci/doc/avis04/Advies_2004-24.pdf)

- 1  
2  
3 334 Atef M, Youssef SAH, El-Eanna HA, El-Maaz AA. 2002. Influence of aflatoxin B1 on the kinetic  
4 335 disposition, systemic availability and tissues residues of doxycycline in chickens. Br Poult Sci.  
5 336 43 (4): 528-532.  
6  
7  
8  
9 337 Baert K, De Baere S, Croubels S, De Backer P. 2003. Pharmacokinetics and oral bioavailability  
10 338 of sulfadiazine and thrimetoprim in broiler chickens. Vet Res Commun. 27: 301-309.  
11  
12  
13 339 Borrás S, Companyo R, Granados M, Guiteras J, Perez-Vendrell AM, Brufau J, Medina M and  
14 340 Bosch J. 2011. Analysis of antimicrobial agents in animal feed. Trends Analyt Chem. 30(7):  
15 341 1042-1064.  
16  
17  
18  
19 342 BCFI. 2010. Gecommentarieerd Geneesmiddelenrepertorium voor Diergeneeskundig  
20 343 gebruik. Brussel. Belgisch Centrum voor Farmacotherapeutische Informatie.  
21  
22  
23  
24 344 Castanon JIR. 2007. History of the use of antibiotic as growth promoters in European poultry  
25 345 feeds. Poult Sci. 86: 2466-2471.  
26  
27  
28 346 Cherlet M, Schelkens M, Croubels S, De Backer P. 2003. Quantitative multi-residue analysis  
29 347 of tetracyclines and their 4-epimers in pig tissues by high-performance liquid  
30 348 chromatography combined with positive-ion electrospray ionization mass spectrometry.  
31 349 Anal Chim Acta. 492: 199-213.  
32  
33  
34  
35 350 Daniel P. 2009. Drugs and chemicals in aquafeeds: The problems and solutions. Options  
36 351 Méditerranéennes A. 86: 85-94.  
37  
38  
39  
40 352 Donoghue DJ, Hairston H, Henderson M, McDonald M, Gaines S, Donoghue AM. 1997.  
41 353 Modeling drug residue uptake by eggs: Yolks contain ampicillin residues even after drug  
42 354 withdrawal and nondetectability in the plasma. Poult Sci. 76: 458-462.  
43  
44  
45  
46 355 Donoghue DJ. 2003. Antibiotic residues in poultry tissues and eggs: Human health concerns?  
47 356 Poult Sci. 82: 618-621.  
48  
49  
50  
51 357 European Commission. 2001. Directive 2001/82/EC of the European parliament and of the  
52 358 Council on the community code relating to veterinary medicinal products. Off. J. Eur. Union.  
53 359 L311: 1-66.  
54  
55  
56  
57  
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1  
2  
3 360 European Commission. 2002. Regulation (EC) No 178/2002 of the European parliament and  
4 361 of the Council laying down the general principles and requirements of food law, establishing  
5 362 the European Food Safety Authority and laying down procedures in matters of food safety.  
6  
7 363 Off. J. Eur. Union. L31: 1-24.

8  
9  
10  
11 364 EMEA. Committee for veterinary medicinal products sulfonamides (2) summary report  
12 365 [Internet]. 1995. London (UK): The European Agency for the Evaluation of Medicinal  
13 366 products veterinary Medicines Evaluation Unit; [cited 1995]. Available  
14 367 from:<http://www.ema.europa.eu/>

15  
16  
17  
18 368 EMEA. Committee for veterinary medicinal products doxycycline hyclate summary report  
19 369 [Internet]. June 1996. London (UK): The European Agency for the Evaluation of Medicinal  
20 370 Products Veterinary Medicines Evaluation Unit; [cited 1996 Jun]. Available  
21 371 from:<http://www.ema.europa.eu/>

22  
23  
24  
25  
26 372 EMEA. Additional Quality requirements for products intended for incorporation into animal  
27 373 feedingstuffs (medicated premixes). [Internet]. December 1996. London (UK): The european  
28 374 agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit; [cited  
29 375 1996 Dec]. Available from:<http://www.ema.europa.eu/>.

30  
31  
32  
33  
34 376 EFSA. Cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a  
35 377 feed additive. [Internet]. 2007. The European Food Safety Authority Journal; [cited 2007 Sept  
36 378 20]. Available from: <http://www.efsa.europa.eu/>

37  
38  
39  
40  
41  
42 380 Ismail MM, El-Kattan YA. 2004. Disposition kinetics of doxycycline in chickens naturally  
43 381 infected with *Mycoplasma gallisepticum*. Br Poult Sci. 45(4): 550-556.

44  
45  
46 382 Kan CA, Petz M. 1999. Residues in veterinary drugs in eggs and their distribution between  
47 383 egg white and yolk. Paper presented at: Symposium on Agrochemical Residues in Eggs.  
48 384 National Meeting of the American-Chemical –Society; 1999; New Orleans, Louisiana.

49  
50  
51  
52 385 Kan CA and Petz M. 2000. Residues of veterinary drugs in eggs and their distribution  
53 386 between yolk and white. J Agric Food Chem. 48(12):6397-6403.



- 1  
2  
3 387 Laczay P, Semjén G, Lehel J, Nagy G. 2001. Pharmacokinetics and bioavailability of doxycycline  
4 388 in fasted and non fasted broiler chickens. *Acta Vet Hung.* 49 (1):31-7.  
5  
6  
7 389 Mortier L, Daeseleire E, Huyghebaert G, Grijspeerdt K, van Peteghem C. 2005. Detection of  
8 390 residues of the coccidiostat diclazuril in poultry tissues by liquid chromatography-tandem  
9 391 mass spectrometry after withdrawal of medicated feed. *J Agric Food Chem.* 53:905-911.  
10 392  
11  
12  
13  
14 393 Reyes-Herrera I, Schneider MJ, Cole K, Farnell MB, Blore PJ, Donoghue DJ. 2005.  
15 394 Concentrations of antibiotic residues vary between different edible muscle tissues in poultry.  
16 395 *J Food Prot.* 68 (10): 2217-2219.  
17  
18  
19  
20 396 Reyes-Herrera I, Donoghue DJ. 2008. Antibiotic residues distribute uniformly in broiler  
21 397 chicken breast muscle tissue. *J Food Prot.* 71 (1): 223-225.  
22  
23  
24  
25 398 Roudaut B and Garnier M. 2002. Sulphonamide residues in eggs following drug  
26 399 administration via the drinking water. *Food Addit Contam.* 19(4):373-378.  
27  
28  
29 400 Santos MDF, Vermeersch H, Remon JP, Schelkens M, De Backer P, Ducatelle R, Haesebrouck  
30 401 F. 1997. Administration of doxycycline hydrochloride via drinking water to turkeys under  
31 402 laboratory and field conditions. *Poult Sci.* 76:1342-1348.  
32  
33  
34  
35 403 Sasanya JJ, Okeng JWO, Ejobi F, Muganwa M. 2005. Use of sulfonamides in layers in Kampala  
36 404 district, Uganda and sulfonamide residues in commercial eggs. *Afr Health Sci* 5 (1): 33-39.  
37  
38  
39 405 Segato G, Bennett C, Angeletti R, Montesissa C, Biancotto G. 2011. Doxycycline and  
40 406 sulfadimethoxine transfer from cross-contaminated feed to chicken tissues. *Food Addit.*  
41 407 *Contamin.* 28(7): 860-868.  
42  
43  
44  
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46 408  
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1  
2  
3 409 Toutain PL, Lees P. 2004. Integration and modelling of pharmacokinetic and  
4 410 pharmacodynamic data to optimize dosage regimens in veterinary medicine. J Vet  
5 411 Pharmacol Ther. 27(6):467-477.

6  
7  
8  
9 412 Usansky JI, Desai A, Tang-Liu D. PK functions for Microsoft Excel. Department of  
10 413 Pharmacokinetics and Drug Metabolism. Allergan, Irvine, CA 92606, USA.

11  
12  
13 414 Vincent U, Ezerskis Z, Chedin M, von Holst C. 2011. Determination of ionophore coccidiostats  
14 415 in feeding stuffs by liquid chromatography-tandem mass spectrometry. Part II. Application to  
15 416 cross-contamination levels and non-targeted feed. J Pharm Biomed Anal. 54: 526-534

16  
17  
18  
19 417 Yoshimura H, Osawa N, Rasa FSC, Hermawati D, Werdiningsih S, Isriyanthi NMR, Sugimori T.  
20 418 1991. Residues of doxycycline and oxytetracycline in eggs after medication via drinking  
21 419 water to laying hens. Food Addit Contam. 8 (1):65-69.

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## 27 28 421 **Tables**

29  
30 422 Table 1: Mean and standard deviation ( $\mu\text{g kg}^{-1}$ ) of the pooled sample and the six individual  
31 423 chickens on day 13 of the treatment period.

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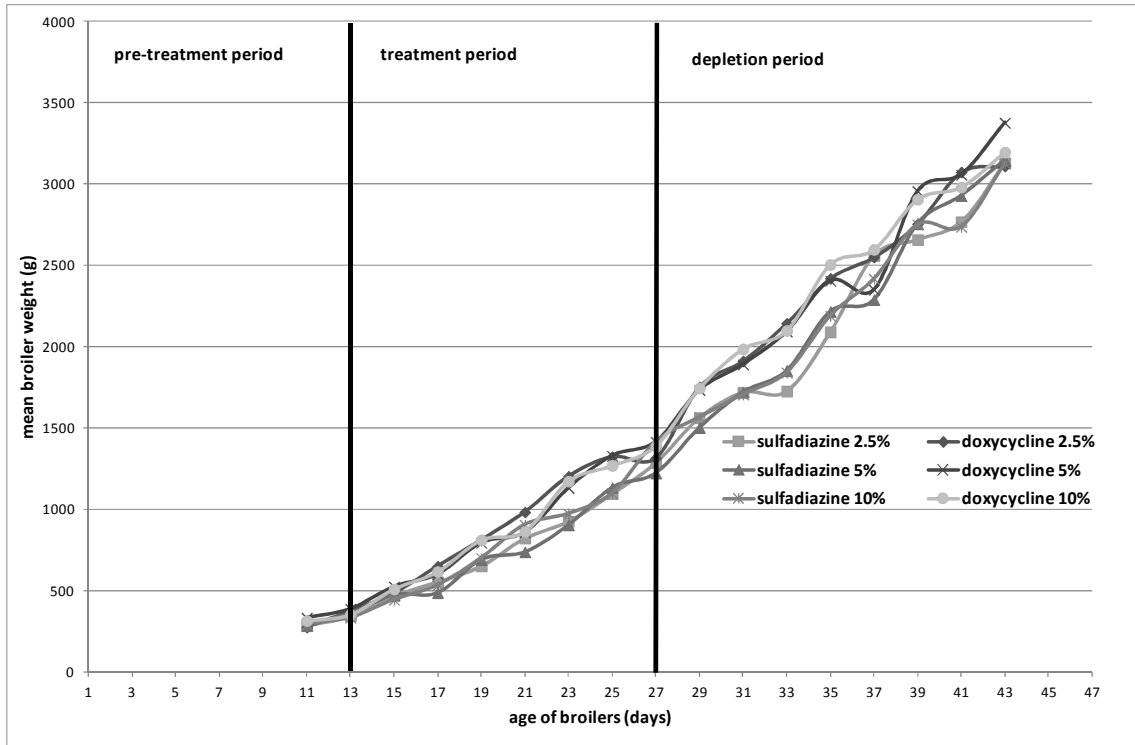
## 35 36 425 **Figures**

37  
38 426 Figure 1: Mean broiler weight (n= 6/conc. group) of the 3 concentration groups of the  
39 427 sulfadiazine and doxycycline study during pre-treatment period (day 1 -> day 12 included),  
40 428 treatment period (day 13 -> day 26 included) and depletion period (day 27 -> day 43).

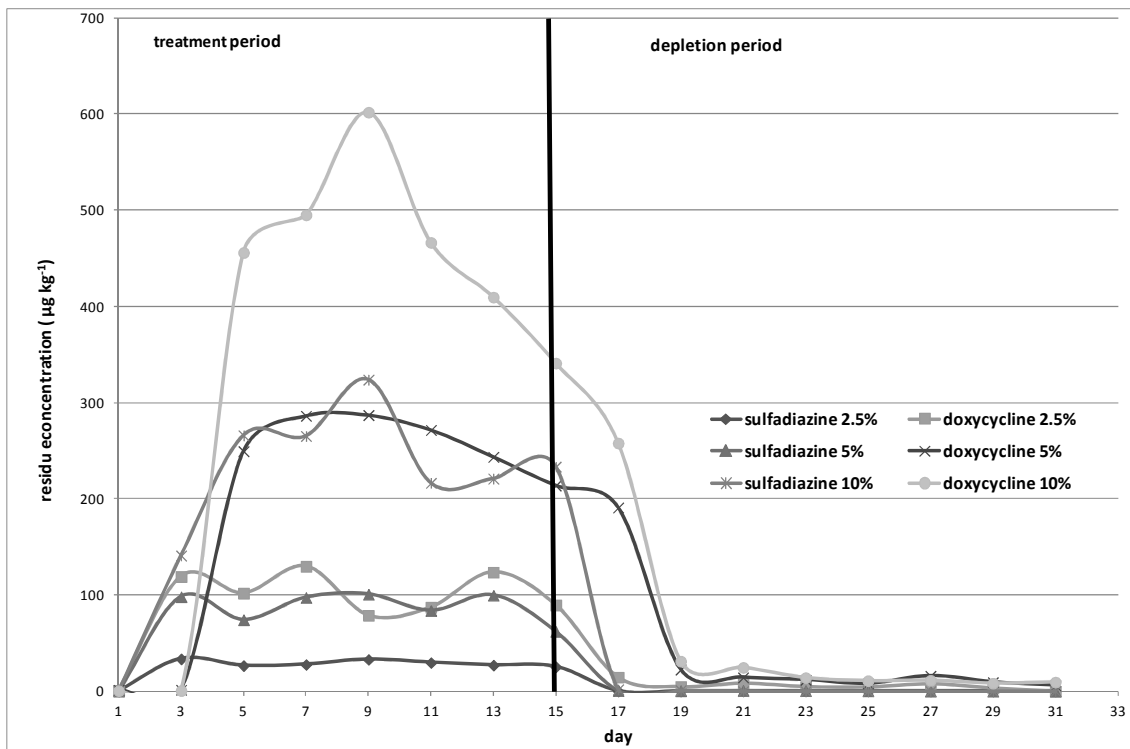
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43 429 Figure 2: Residue concentrations of the 3 sulfadiazine and doxycycline concentration groups  
44 430 (2.5; 5 and 10 %) in the breast muscle during treatment period (day 1 -> day 14 included)  
45 431 and depletion period (day 15 -> day 31).

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48 432 Figure 3: Residue concentration in breast muscle, thigh muscle and liver for sulfadiazine 10%  
49 433 and doxycycline 10 % during treatment period (day 5 -> day 14 included) and depletion  
50 434 period (day 15).

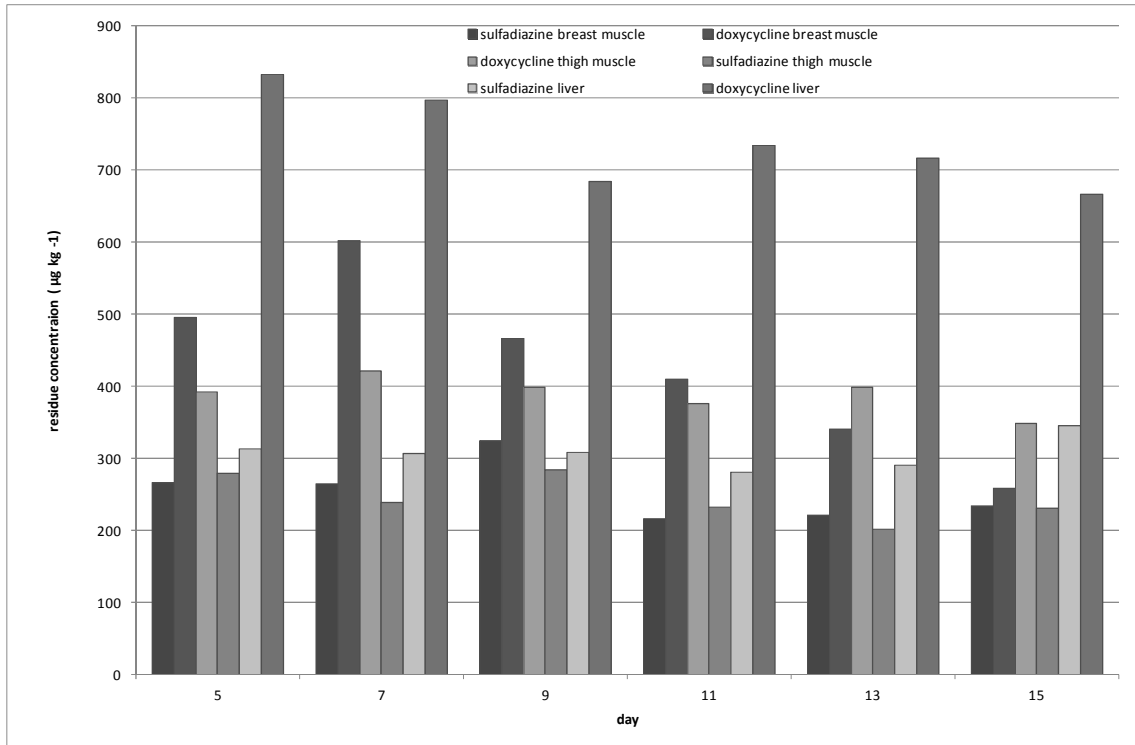
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Molecule	Sample type	Matrix	Mean and standard deviation ( $\mu\text{g kg}^{-1}$ )
Sulfadiazine	Six analyses of the pooled sample	Breast muscle	201.6 $\pm$ 15.7
		Thigh muscle	172.2 $\pm$ 7.7
		Liver	358.0 $\pm$ 20.4
	Analysis of six individual chickens	Breast muscle	202.6 $\pm$ 49.8
		Thigh muscle	173.1 $\pm$ 30.3
		Liver	355.8 $\pm$ 109.0
Doxycycline	Six analyses of the pooled sample	Breast muscle	485.7 $\pm$ 88.8
		Thigh muscle	344.4 $\pm$ 13.3
		Liver	728.4 $\pm$ 74.4
	Analysis of six individual chickens	Breast muscle	474.8 $\pm$ 107.5
		Thigh muscle	327.7 $\pm$ 32.7
		Liver	726.2 $\pm$ 74.7

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