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## Sulfadimidine as a model to evaluate pharmacokinetics and residues at various concentrations in laying hen

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## Abstract

A low level intake of drugs from the ingestion of contaminated feed may lead to residue problems in food animals. Sulfadimidine (SDD) was used as a model to determine the residue risk at various doses in laying hens. The drug was administered as a single intravenous injection (100 mg kg<sup>-1</sup> body weight, BW), as a single oral dose (100, 30, 10, 3, 1 mg kg<sup>-1</sup> BW) and via medicated feed for seven consecutive days (30, 10, 3 mg kg<sup>-1</sup> BW). Drug levels were determined with HPLC-UV for plasma, yolk and albumen. Pharmacokinetic values, which were calculated using a first-order one-compartment model, residue levels and transfer rates into the eggs, were found to be dose-dependent. Even low doses at 3 and 1 mg kg<sup>-1</sup> BW resulted in measurable residues in yolk and albumen one day after a single oral administration. After ingestion of medicated feed at 3 mg kg<sup>-1</sup> BW mean drug levels at  $0.14 \pm 0.01 \mu\text{g g}^{-1}$  were found in albumen and at  $0.09 \pm 0.01 \mu\text{g ml}^{-1}$  in plasma. Generally, the residue levels in albumen and plasma were higher than in yolk. These findings demonstrate a residue risk for the consumer even after low level intake of drugs.

**Keywords:** sulfadimidine, laying hen, pharmacokinetic, residue.

## Introduction

Antibiotics and coccidiostats are of widespread use in food animals. Improper use or cross-contamination during manufacture of animal feed with these compounds may cause residue problems. Cross-contamination of feed is a well-known problem and may be due to an improper construction of the equipment, poor dust control or inadequate clean-out procedures between sequential batches of feed (FDA 2005). Even very low levels of antibiotic contamination may result in relevant residues in food, as confirmed by studies on coccidiostats in poultry feed (Rokka et al. 2005, Kennedy et al. 1998) and sulfadimidine in swine (Biehl et al. 1981, McCaughey et al. 1990). Sulfonamides are veterinary drugs routinely used in farm animals, and sulfonamide residues in animal products such as egg and milk have recently been investigated (Furusawa & Kishida 2002, Cavaliere et al. 2003, Juhel-gaugain et al. 2005). We therefore used sulfadimidine in laying hens as a model to study the impact of different dosages of sulfadimidine on its pharmacokinetics and risk of residue build-up in eggs.

Briefly, sulfadimidine (SDD) which is also known as sulfamethazine is one of the drugs regularly used in poultry husbandry for its broad-spectrum activities against bacterial and coccidial infections in chicken. The European Union (EU) and the *Codex Alimentarius* Commission have established a maximum residue limit of sulfonamides (MRL =  $0.1 \mu\text{g g}^{-1}$ ) for many kinds of animal products; however, no MRL is established for eggs. Sulfonamides are therefore unapproved for administration in laying hens (EEC 2377/90, *Codex Alimentarius*).

Previous studies, pharmacokinetic behaviors of SDD in chicken have been described (Kietzmann 1980, Geertsma et al. 1987, Nouws et al. 1988, and Jian & Punia, 2001). Different doses and routes of SDD residues have also been studied in laying hen (Blom 1975, Seib 1991, Roudaut & Garnier 2002, Furusawa 2001, Kan 2003). These studies indicated a transfer of sulfonamides into the eggs, but mainly provided data focusing on therapeutic doses. Therefore, information about the pharmacokinetic behaviors and residue risk following the administration of SDD at low doses are scarce. As the extrapolation of high to low-level kinetics is generally regarded to be inadequate (Braun et al. 1983) this study was conducted to clarify the residue risk after low-level ingestion of sulfadimidine in laying hens.

## Materials and methods

*Animals:* Healthy laying hens (Lohmann Brown), 22-26 weeks of ages ( $1.95 \pm 0.15$  kg), were housed at controlled temperature ( $20 \pm 2^\circ\text{C}$ ) and light ( $14 \text{ h day}^{-1}$ ) in accordance with the animal welfare acts (33-42502-04/917, LAVES). Water and a commercial standard laying hen mash (deuka Deutsche Tiernahrung, Düsseldorf, Germany) were supplied *ad libitum*.

*Reagents and chemicals:* All reagents and chemicals used were of HPLC or analytical grade. Acetonitrile, ethyl acetate and n-hexane were obtained from Labscan (Dublin, Ireland). Phosphate hydrogen monohydrate, sodium chloride, hydrochloric acid (Merck, Germany) and 85% acetic acid (AppliChem, Germany) were used. A  $0.05 \text{ mol l}^{-1}$

1 phosphate buffer, pH 3, was prepared by dissolving 0.68 g of phosphate monohydrate with  
2 1000 ml of high purity water, and then adjusted to pH 3 with 85% acetic acid.

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4  
5 *Standards:* Sulfadimidine (SDD) and sulfadoxine (SDX) were purchased from Sigma-  
6 Aldrich (Steinheim, Germany). SDX, as internal standard, was dissolved in methanol at  
7 concentrations of 50  $\mu\text{g ml}^{-1}$ . SDD standard solutions were prepared in distilled water at  
8 concentration from 1 to 0.001  $\text{mg ml}^{-1}$ .

9  
10  
11 *Drug administration and sample collection:* Groups of six hens each were treated with  
12 SDD via intravenous injection (i.v., 1 group), via a single bolus oral administration (p.o., 7  
13 groups), or via medicated feed (3 groups). For the i.v. group SDD was injected as a single  
14 bolus via the vena axillaris at 100  $\text{mg kg}^{-1}$  body weight (BW). For the oral administration  
15 of a single dose, 100, 30, 10, 3, 1  $\text{mg kg}^{-1}$  BW were used. The three groups receiving  
16 medicated feed were treated for seven consecutive days with mixed feed resulting in a dose  
17 of 30, 10 and 3  $\text{mg kg}^{-1}$  BW per day. For the analysis, blood samples of 1.5 ml were taken  
18 from the brachial vein at pre-determined intervals and heparinised to prevent blood  
19 clotting. These were centrifuged at 4100 g for 10 min to separate the plasma, which was  
20 placed in a 2 ml micro centrifuge vial for drug extraction. Eggs were collected for 7 days,  
21 starting 20 h after the treatment. Egg yolk and egg albumen were separated, and placed into  
22 10 ml polypropylene test tubes. All samples were stored at  $-20^{\circ}\text{C}$  pending analysis.

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26 *Plasma extraction:* 10  $\mu\text{l}$  of SDX solution ( $50\mu\text{g ml}^{-1}$ ) were added to 490  $\mu\text{l}$  of plasma  
27 along with 100  $\mu\text{l}$  of 0.1 N HCl and 1 ml of ethyl acetate. After mixing in a vortex mixer  
28 for 1 min and centrifugation at 23100 g for 5 min, 600  $\mu\text{l}$  of the supernatant were  
29 evaporated under a gentle  $\text{N}_2$  stream at  $40^{\circ}\text{C}$ .

30  
31  
32 *Yolk extraction:* 1 g of yolk was weighed into a 10 ml test tube. The sample was mixed  
33 with 2.9 ml distilled water and internal standard (10  $\mu\text{l}$  of  $100\mu\text{g ml}^{-1}$  of SDX).  
34 Sulfonamides were extracted after adding 0.5 ml of 0.1 N HCl and 2 ml of ethyl acetate by  
35 mixing a vortex mixer for 2 min. An excessive amount of NaCl (approximately 1.2 g), 100  
36  $\mu\text{l}$  of 1 N HCl and 500  $\mu\text{l}$  of n-hexane were added to break the emulsion. After mixing for 2  
37 min and centrifugation at 4100 g for 5 min, 1.5 ml of the supernatant was taken into a  
38 second 10 ml test tube. The remainder was extracted a second time. For this, 1.5 ml ethyl  
39 acetate were added, then mixed for 2 min and centrifuged at 4100 g for 5 min. 1.5 ml of the  
40 upper phase were combined with the previous one and evaporated to dryness under a gentle  
41 stream of  $\text{N}_2$ .

42  
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44  
45 *Albumen extraction:* A 1 g portion of albumen was weighed into a test tube, and 0.5 ml  
46 distilled water and 10  $\mu\text{l}$  of SDX ( $100\mu\text{g ml}^{-1}$ ) were added along with 0.5 ml of 0.1 N HCl,  
47 0.9 ml of acetonitrile and 0.1 ml of n-hexane. After mixing in a vortex mixer for 2 min,  
48 excessive NaCl (approximately 1.2 g) and 2 ml of ethyl acetate were added. After mixing  
49 for 2 min and centrifugation at 4100 g for 5 min 2 ml of the upper phase were evaporated  
50 under a gentle  $\text{N}_2$  stream at  $40^{\circ}\text{C}$ . All residues were reconstituted in 700  $\mu\text{l}$  of eluent and  
51 centrifuged at 23100 g for 5 min. A 100  $\mu\text{l}$  aliquot of the clear lower phase was injected  
52 into the HPLC system.

53  
54  
55 *HPLC apparatus, conditions and validation:* The HPLC apparatus consisted of an UV  
56 detector; Varian<sup>®</sup> 2050, solvent delivery system; Beckman<sup>®</sup>, programmable solvent  
57 module 116, an auto sampler; Gilson, model 231, chromatographic software; 32-Karat<sup>™</sup>

Software, version 3.0. Separation was achieved on an analytical column (LiChrospher 100 RP-18, endcapped, 125×4, 5 µm; Merck, Germany) and guard cartridge. The temperature was set to 40°C and controlled with a column oven. A mobile phase consisting of 0.05 mol l<sup>-1</sup> phosphate buffer (pH 3.0) and acetonitrile at 85:15, v/v and 89:11, v/v was used for plasma and egg analysis, respectively. A flow rate of 1.5 ml min<sup>-1</sup> and a wavelength of 268 nm were used throughout.

The method was validated according to FDA requirements (FDA 2001) and was found to have a limit of quantification (LOQ) of 0.025 µg ml<sup>-1</sup> for plasma and 0.05 µg g<sup>-1</sup> for both albumen and yolk. The limits of detection (LOD) were established to be 0.01 µg ml<sup>-1</sup> for plasma and 0.02 µg g<sup>-1</sup> for eggs as determined from the calibration curve according to DIN 32645 (1994). As shown in figure 1 no interferences were detected at the retention times of SDD and the internal standard SDX (I.S.). The method was linear in the range between LOQ and 100 µg ml<sup>-1</sup> (µg g<sup>-1</sup>) (R<sup>2</sup>= 0.999). Accuracy, intra- and inter-day precision were determined using quality control samples at concentration of 0.025-0.10 µg ml<sup>-1</sup>. For both plasma and eggs, accuracy as expressed as the relative deviations from expected values was less than 15%. Intra- and inter-day precision were less than 10 % except at the LOQ of plasma (16.9%). The recoveries were 81-94 %, 88-97 % and 90 % for plasma, yolk and albumen, respectively.

*Pharmacokinetic parameters and drug transfer rate calculation:* Pharmacokinetic parameters were calculated using WinNonLin® version 4.1, Pharsight® (USA). A first-order one-compartment model was used for the calculations. The area under the curve (AUC), half life of absorption (K01\_HL), elimination half life (K10\_HL), total body clearance (Cl), time of maximum plasma concentration (T<sub>max</sub>) and maximum plasma concentration (C<sub>max</sub>) were calculated.

For the calculation of the drug transfer rate (TR) into the eggs, the sum of residues (R<sub>total</sub>) in albumen and yolk were calculated using the measured concentrations in (C<sub>albumen</sub>, C<sub>yolk</sub>) and mass of albumen and yolk (m<sub>albumen</sub>, m<sub>yolk</sub>) at day i as:

$$[1] \quad R_{total,i} = c_{albumen,i} \cdot m_{albumen,i} + c_{yolk,i} \cdot m_{yolk,i}$$

For the mass of yolk and albumen, an average weight of six representative eggs was used (13.84 g and 36.28 g, respectively). The transfer rate was calculated from the sum of residues of all eggs measured up to 10 days after administration as:

$$[2] \quad TR[\%] = \frac{\sum_{i=1}^{t=10} R_{total,i}}{dose} \cdot 100$$

## Results

The semi logarithmic plots of plasma concentrations vs. time curves of SDD (Mean ± SD) following i.v. and p.o. application are given in figure 2. Pharmacokinetic parameters were not calculated for the p.o. group following 1 mg kg<sup>-1</sup> BW due to insufficient data quality. SDD was very quickly eliminated from the plasma. Within 6-32 h after p.o. administration

1  
2 at various doses, the concentrations were lower than the LOQ. The pharmacokinetic  
3 parameters calculated assuming first order kinetics are given in table I. After single oral  
4 doses, the  $C_{max}$ ,  $T_{max}$  and the  $K_{10\_HL}$  increased while the  $Cl$  decreased with increasing  
5 doses (see figure 3).  
6

7  
8 [Insert figure 1 and 2 about here]  
9

10 [Insert table I about here]  
11

12 [Insert figure 3 about here]  
13

14  
15 The residue levels in eggs after single oral doses are summarized in tables II and III. In  
16 general, SDD was detectable for longer in yolk than in albumen and plasma. However, in  
17 the initial phases considerably higher concentrations of SDD were measured in albumen  
18 and plasma compared to yolk. In yolk, residues above the LOQ were detectable up to 8  
19 days following the normal therapeutic dose ( $100 \text{ mg kg}^{-1} \text{ BW}$ ). By using lower doses of  
20 30, 10, and  $3 \text{ mg kg}^{-1} \text{ BW}$ , drug levels over the LOQ were measured up to 7, 4 and 1 day  
21 post single oral doses, respectively. No drug levels in yolk above the LOQ were measured  
22 after treatment with  $1 \text{ mg kg}^{-1} \text{ BW}$ . In albumen, drug concentrations above the LOQ  
23 appeared for 6, 3, 1, 1, and 1 days after treatment with 100, 30, 10, 3 and  $1 \text{ mg kg}^{-1} \text{ BW}$ ,  
24 respectively. The calculated transfer rates of SDD into the eggs after single oral doses are  
25 dose dependent (table IV). Decreasing doses are related with a diminishing transfer rate.  
26  
27

28  
29 During treatment with medicated feed for 7 days, approximately equal concentrations were  
30 measured in plasma and albumen but higher than yolk (table V), for groups ingesting 30  
31 and  $10 \text{ mg kg}^{-1} \text{ BW}$ . In the group of  $3 \text{ mg kg}^{-1} \text{ BW}$ , residue levels in albumen exceeded  
32 even in plasma, but concentrations were lower than LOQ in yolk. At all concentrations,  
33 residues rapidly accumulated in eggs, as proved by high concentrations of SDD in eggs  
34 collected 20 h after treatment.  
35

36  
37 [Insert table II about here]  
38

39 [Insert table III about here]  
40

41 [Insert table IV about here]  
42

43 [Insert table V about here]  
44  
45

## 46 47 Discussion

48  
49 We used sulfadimidine as a model to estimate the risk of a residue build-up in eggs after  
50 unintended low-level intake. At the therapeutic dose, pharmacokinetic values in this present  
51 study consistent with former studies (Geertsma et al. 1987, Nouws et al. 1988, and Jian &  
52 Punia 2001). In general, SDD is known to be rapidly absorbed (Botsoglou & Fletouris  
53 2001) and quickly eliminated (Nouws et al. 1988) after oral therapy. At low doses,  
54 however, our data demonstrate that SDD is absorbed even more rapidly in a dose  
55 dependent manner, resulting in a decrease of the calculated absorption rate half-life  
56 ( $K_{01\_HL}$ ) to 0.2 h at  $3 \text{ mg kg}^{-1} \text{ BW}$  as compared to 1.2 h at  $100 \text{ mg kg}^{-1} \text{ BW}$ .  
57  
58  
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60

Correspondingly, the  $T_{\max}$  value decreases from 4 h to 0.5 h. Furthermore, decreasing doses are associated with larger volumes of total body clearance resulting in dose dependent shorter elimination half-life, which decrease from 9.1 h for 100 mg kg<sup>-1</sup> BW to 0.9 h for 3 mg kg<sup>-1</sup> BW. Rate-limited absorption may explain the deviations from dose independent total clearance, which is expected using first order kinetics. However, the nearly equal clearance after i.v. and p.o application with the same dose (100 mg kg<sup>-1</sup> BW) suggests that in fact also the excretion is rate-limited, with both processes resulting in over- proportional decrease of the AUC after decreasing doses of SDD. The underlying physiologic mechanisms involved in this complex pattern of absorption and excretion of SDD in chicken remain to be clarified. The limited data gathered in this study however do not allow for further interpretations or the application of more complex pharmacokinetic models. Nevertheless, as first order kinetics may not be readily assumed for SDD in chicken, pharmacokinetic parameters formerly established on therapeutic levels can not be extrapolated on subtherapeutic levels. The impact of this on residue levels in eggs is clearly reflected by the transfer rate of SDD, which decreases correlating with the dose.

Our data nevertheless demonstrate that measurable residue levels may occur in eggs even after a single low-level intake at only 1% of the recommended dose. Moreover, during ingestion of feed supplemented with 3% of the therapeutic dose, these residue levels are higher than the MRL of 0.1 µg g<sup>-1</sup> established for other tissues throughout.

Interestingly, the accumulation of SDD in different compartments changes with time. According to our results, the SDD concentration at initial phases after single oral administrations and throughout medication via feed is 2 to 4 times higher in albumen than in yolk. However, after single oral doses the SDD level in yolk exceeded that in albumen after about 2 days, supporting previous studies (Geertma et al. 1987, Shaikh & Chu 2000). In addition, SDD residues in yolk were detectable longer after a single oral administration of the therapeutic dose (up to the 8<sup>th</sup> day as compared to the 6<sup>th</sup> day in albumen, whereas it was eliminated from plasma after only 32 h). We therefore confirm the use of yolk as appropriate matrix for monitoring SDD residues as previously suggested for sulfaquinoxaline (Shaikh et al. 2004). The reason for this complex pattern of time depended selective accumulation in certain compartments remains unclear, as recently concluded by Kan (2003), who described none of the formerly studied physicochemical substance properties like pKa value, lipid solubility and protein binding to be clearly linked to the pattern of distribution of sulfonamides between plasma, yolk and albumen.

In conclusion, our study demonstrates the existence of a residue risk due to a low level intake of SDD, as the ingestion of the drug at only 1% of the therapeutic dose resulted in measurable concentrations in eggs. The finding of a clear dose dependence of its pharmacokinetics is of special interest for other substances, as it clarifies, that residue risks should be examined at relevant concentrations and may not be estimated from known high-level kinetics.

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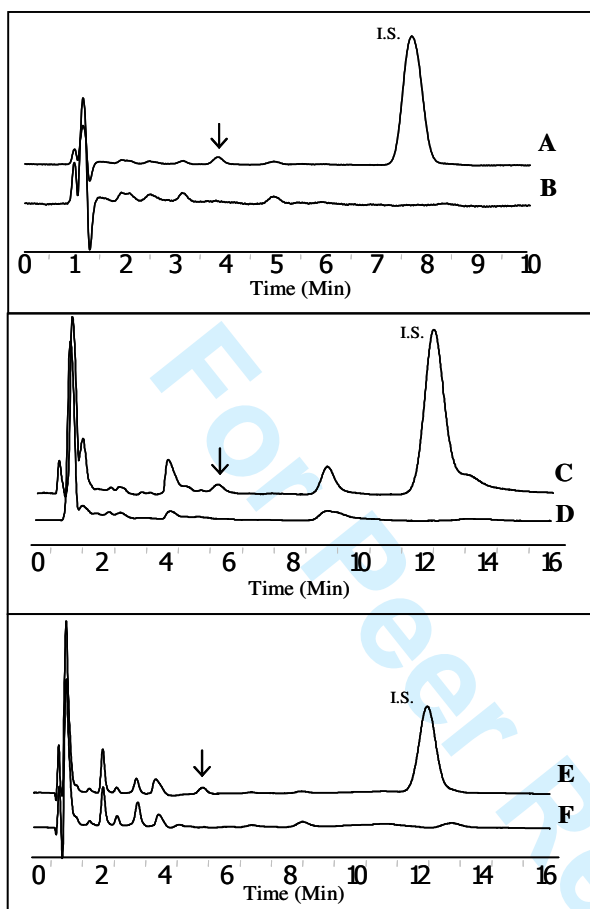
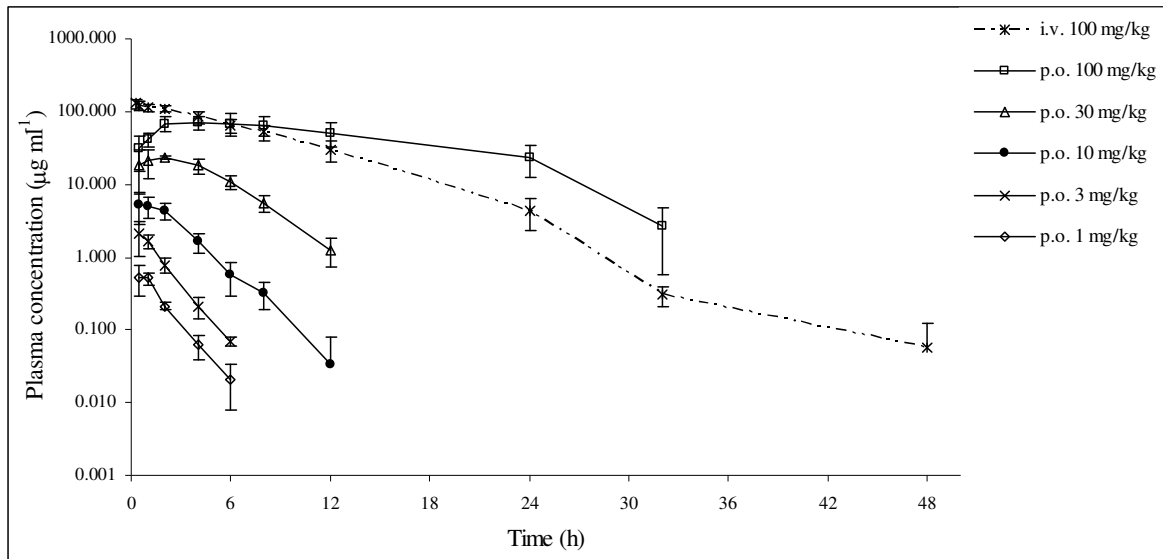


Figure 1. Typical chromatogram obtained from control plasma (B), albumen (D) and yolk (F), respectively. Parts A, C and E show typical chromatogram of fortified plasma ( $25 \text{ ng mL}^{-1}$ ), albumen and yolk ( $50 \text{ ng g}^{-1}$ ), respectively. Black arrow indicate peak of sulfadimidine (I.S.= internal standard).

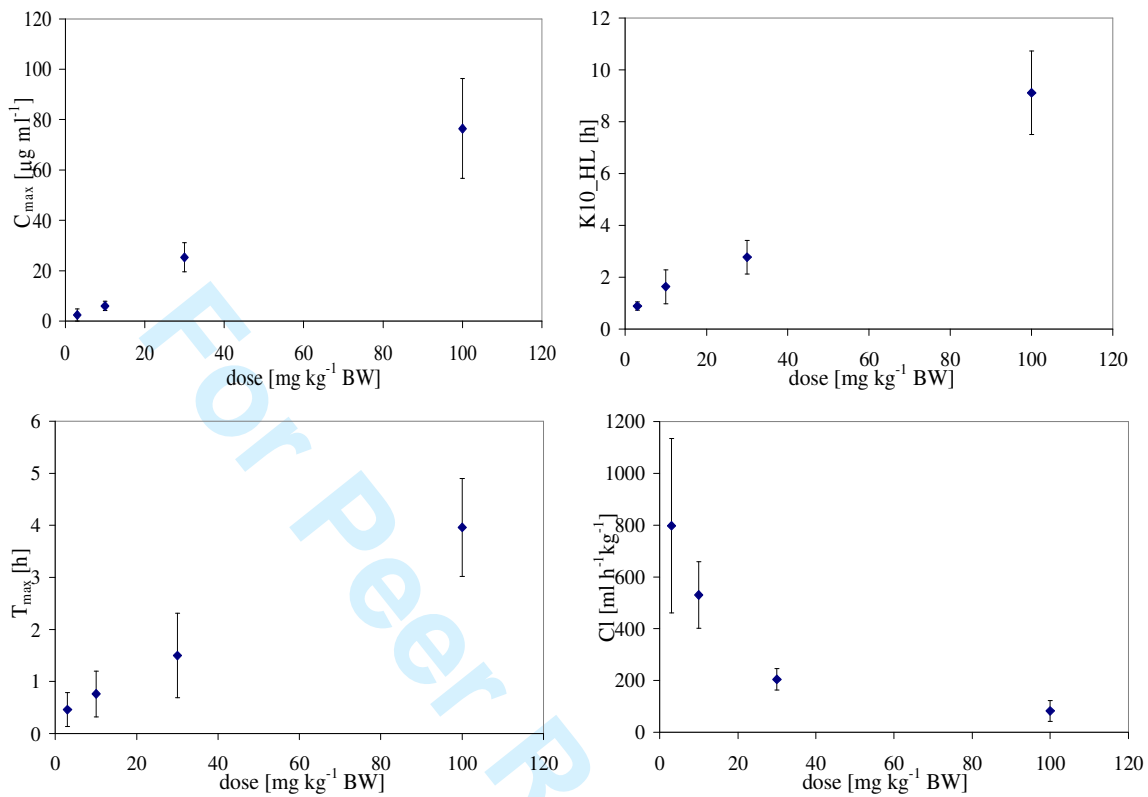


**Figure 2.** Plasma concentrations of sulfadimidine (Mean  $\pm$  SD) versus time after a single intravenous (i.v.) and oral (p.o.) administration (n=6).

Table I. Pharmacokinetic characteristics of sulfadimidine in plasma after single intravenous (i.v.) and oral (p.o.) administration in laying hen (Mean  $\pm$  SD, n=6).

Dose mg kg <sup>-1</sup>	AUC $\mu\text{g h ml}^{-1}$	K01_HL h	K10_HL h	Cl ml h <sup>-1</sup> kg <sup>-1</sup>	T <sub>max</sub> h	C <sub>max</sub> $\mu\text{g ml}^{-1}$
100 (i.v.)	1122.0 $\pm$ 245.8	-	5.9 $\pm$ 1.1	94.1 $\pm$ 27.8	-	132.2 $\pm$ 16.9
100 (p.o.)	1379.3 $\pm$ 428.1	1.2 $\pm$ 0.4	9.1 $\pm$ 1.6	82.5 $\pm$ 40.0	4.0 $\pm$ 0.9	76.5 $\pm$ 19.9
30 (p.o.)	151.5 $\pm$ 27.5	0.7 $\pm$ 0.6	2.8 $\pm$ 0.7	204.2 $\pm$ 41.6	1.5 $\pm$ 0.8	25.3 $\pm$ 5.8
10 (p.o.)	18.5 $\pm$ 4.1	0.4 $\pm$ 0.4	1.4 $\pm$ 0.8	563.2 $\pm$ 119.2	0.7 $\pm$ 0.5	6.2 $\pm$ 1.8
3 (p.o.)	4.2 $\pm$ 1.2	0.2 $\pm$ 0.3	0.9 $\pm$ 0.2	797.3 $\pm$ 337.1	0.5 $\pm$ 0.3	2.4 $\pm$ 1.1

AUC, area under the curve; K01\_HL, half life of absorption; K10\_HL, elimination half life; Cl, total body clearance; T<sub>max</sub>, time of maximum plasma concentration; C<sub>max</sub>, maximum plasma concentration.



**Figure 3.** C<sub>max</sub>, K<sub>10\_HL</sub>, T<sub>max</sub> and Cl values of SDD in plasma after single oral administration of different doses (Mean ± SD, n=6).

Table II. Sulfadimidine residues in yolk ( $\mu\text{g g}^{-1}$ , Mean  $\pm$ SD) after single oral administration of different doses.

Dose ( $\text{mg kg}^{-1}$ BW)	100	30	10	3	1
Day after treatment					
1	$14.32 \pm 6.47^*$	$3.08 \pm 1.52^*$	$0.42 \pm 0.08$	$0.11 \pm 0.05$	ND
2	$6.62 \pm 2.72^*$	$0.51 \pm 0.01^{\ddagger}$	$0.07 \pm 0.02$	< 0.05	ND
3	$3.67 \pm 1.17^*$	$0.44 \pm 0.12^*$	$0.06 \pm 0.02^*$	ND	ND
4	$2.74 \pm 0.69^*$	$0.32 \pm 0.05^{\dagger}$	< 0.05	ND	ND
5	$1.98 \pm 0.56^*$	$0.20 \pm 0.04^{\ddagger}$	< 0.05	ND	ND
6	$1.21 \pm 0.59^*$	$0.13 \pm 0.02^*$	ND	ND	ND
7	$0.43 \pm 0.32^*$	$0.07 \pm 0.03^{\ddagger}$	ND	ND	ND
8	$0.12 \pm 0.14^{\ddagger}$	< 0.05	ND	ND	ND
9	< 0.05	ND	ND	ND	ND
10	ND	ND	ND	ND	ND

n=6 unless otherwise indicated while \*, † and ‡ = 5, 4, 3 eggs laid and analyzed, respectively. ND = not detected (< LOD)

Table III. Sulfadimidine residues in albumen ( $\mu\text{g g}^{-1}$ , Mean  $\pm$ SD) after single oral administration of different doses.

Dose ( $\text{mg kg}^{-1}$ BW)	100	30	10	3	1
Day after treatment					
1	$49.34 \pm 7.03^*$	$6.25 \pm 1.85^*$	$0.98 \pm 0.24$	$0.28 \pm 0.12$	$0.07 \pm 0.02$
2	$6.10 \pm 0.43^*$	$0.16 \pm 0.04^\ddagger$	ND	ND	ND
3	$0.83 \pm 0.16^*$	$0.15 \pm 0.14^*$	ND	ND	ND
4	$0.38 \pm 0.12^*$	ND	ND	ND	ND
5	$0.20 \pm 0.07^*$	ND	ND	ND	ND
6	$0.12 \pm 0.03^*$	ND	ND	ND	ND
7	< 0.05	ND	ND	ND	ND
8	ND	ND	ND	ND	ND

n=6 unless otherwise indicated while \*, †, ‡ = 5, 4, 3 eggs laid and analyzed, respectively. ND = not detected (< LOD)

TableIV. Transfer rate of SDD into eggs after single oral administration.

Dose (mg kg <sup>-1</sup> BW)	Drug intake <sup>a</sup> (mg)	Residue in whole egg <sup>b</sup> (µg)	Transfer-rate <sup>c</sup> (%)
100	195	2497.16	1.28
30	61.5	303.60	0.49
10	19.1	43.17	0.23
3	5.8	11.68	0.20
1	1.8	2.54	0.14

<sup>a</sup> calculated from dose × average BW, (data not shown)

<sup>b</sup> calculated according to equation [1]

<sup>c</sup> calculated according to equation [2]

Table V. Residue concentration of Sulfadimidine (Mean $\pm$  SD) in plasma, albumen and yolk following medicated feed of different doses (30, 10 and 3 mg kg<sup>-1</sup> BW) for seven consecutive days.

Matrix Dose (mg kg <sup>-1</sup> BW)	Plasma ( $\mu\text{g ml}^{-1}$ )			Albumen ( $\mu\text{g g}^{-1}$ )			Yolk ( $\mu\text{g g}^{-1}$ )		
	30	10	3	30	10	3	30	10	3
Day 1	4.87 $\pm$ 2.05	0.58 $\pm$ 0.17	0.11 $\pm$ 0.03	2.50 $\pm$ 1.10	0.42 $\pm$ 0.07	0.13 $\pm$ 0.03 <sup>‡</sup>	0.26 $\pm$ 0.14	<0.05	<0.05
Day 2	3.33 $\pm$ 1.97	0.52 $\pm$ 0.11	0.09 $\pm$ 0.03	5.40 $\pm$ 2.58 <sup>*</sup>	0.62 $\pm$ 0.15	0.15 $\pm$ 0.02	1.23 $\pm$ 0.52 <sup>*</sup>	0.10 $\pm$ 0.03	<0.05
Day 3	4.70 $\pm$ 1.88	0.47 $\pm$ 0.07	0.09 $\pm$ 0.02	4.86 $\pm$ 1.44	0.61 $\pm$ 0.15	0.15 $\pm$ 0.01	1.50 $\pm$ 0.70	0.13 $\pm$ 0.03 <sup>*</sup>	<0.05
Day 4	3.99 $\pm$ 1.52	0.44 $\pm$ 0.05	0.09 $\pm$ 0.03	4.99 $\pm$ 1.93 <sup>*</sup>	0.64 $\pm$ 0.05 <sup>*</sup>	0.14 $\pm$ 0.01	2.04 $\pm$ 0.60 <sup>*</sup>	0.15 $\pm$ 0.03	<0.05
Day 5	2.36 $\pm$ 2.41	0.43 $\pm$ 0.07	0.10 $\pm$ 0.03	3.77 $\pm$ 1.25 <sup>*</sup>	0.45 $\pm$ 0.06	0.13 $\pm$ 0.02 <sup>*</sup>	1.80 $\pm$ 0.52 <sup>*</sup>	0.16 $\pm$ 0.02	<0.05
Day 6	2.91 $\pm$ 1.08	0.31 $\pm$ 0.10	0.07 $\pm$ 0.01	2.84 $\pm$ 1.25 <sup>*</sup>	0.40 $\pm$ 0.12	0.14 $\pm$ 0.02	1.55 $\pm$ 0.45 <sup>*</sup>	0.20 $\pm$ 0.02	<0.05
Day 7	1.48 $\pm$ 0.95	0.37 $\pm$ 0.12	0.09 $\pm$ 0.02	2.97 $\pm$ 0.90 <sup>†</sup>	0.43 $\pm$ 0.14	0.13 $\pm$ 0.01	1.64 $\pm$ 0.55 <sup>†</sup>	0.20 $\pm$ 0.02	<0.05

\*, †, ‡ 5, 4 and 3 eggs laid and analyzed, respectively

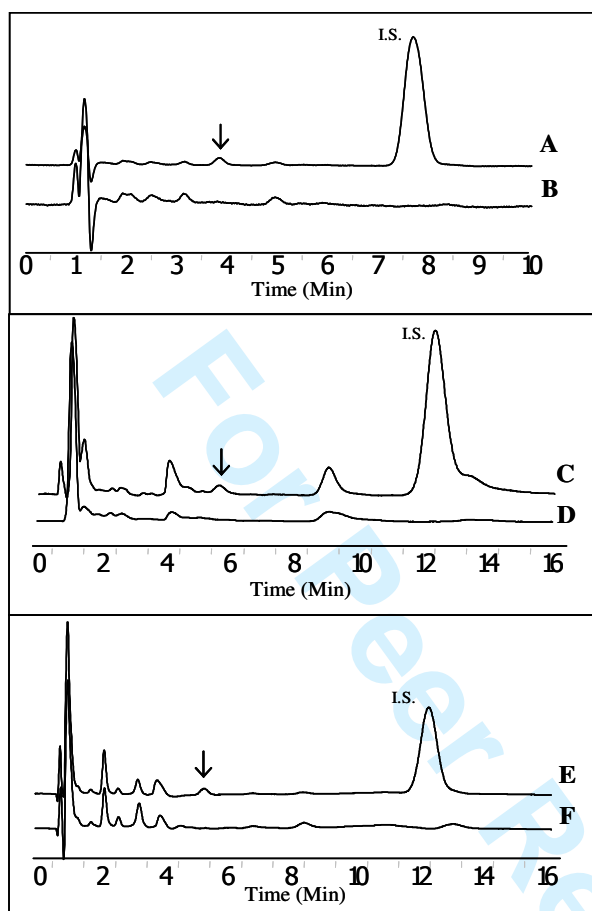
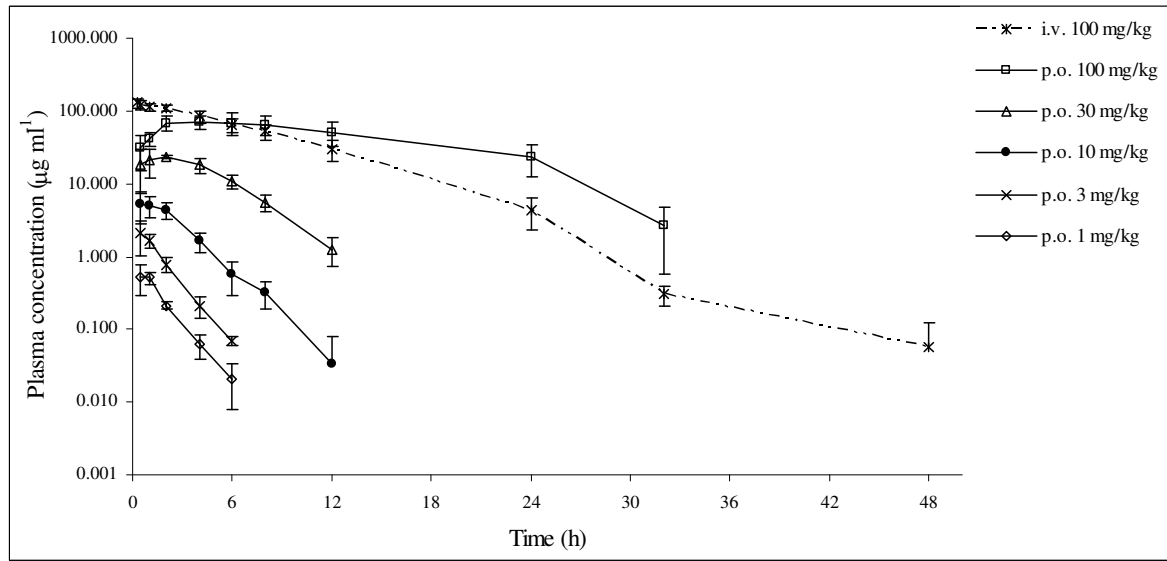
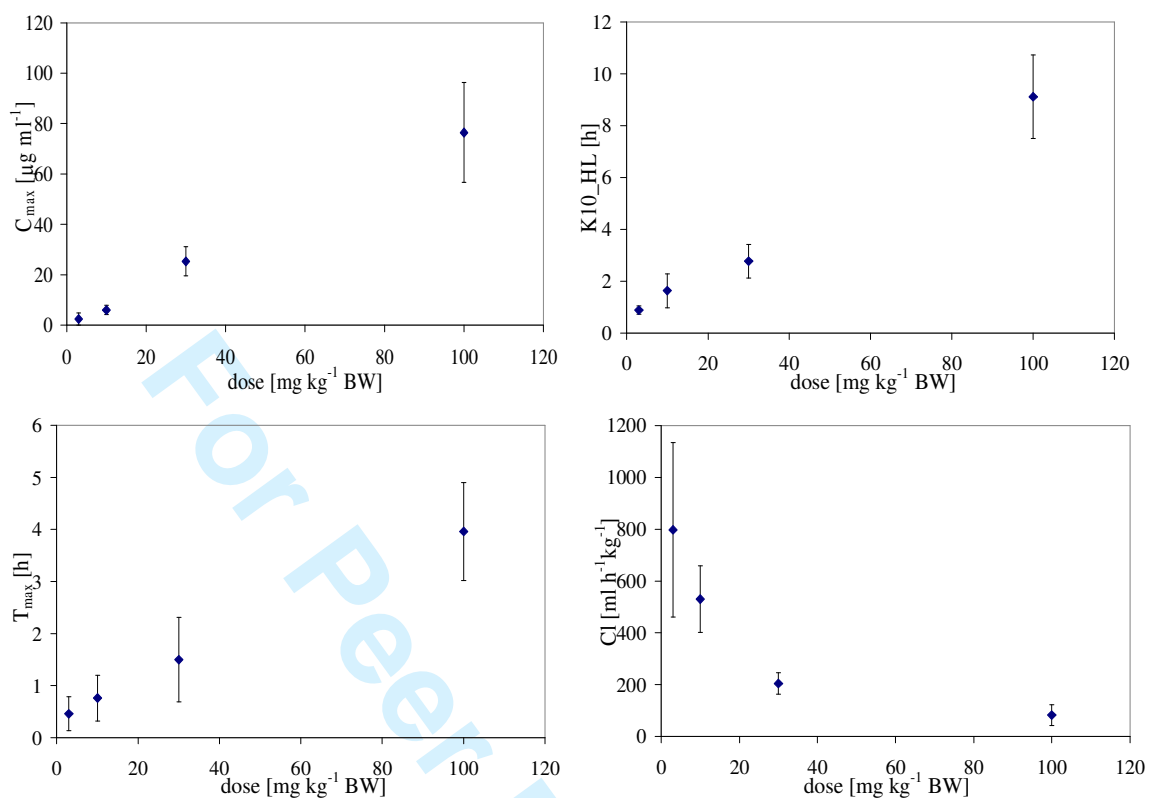


Figure 1. Typical chromatogram obtained from control plasma (B), albumen (D) and yolk (F), respectively. Parts A, C and E show typical chromatogram of fortified plasma ( $25 \text{ ng mL}^{-1}$ ), albumen and yolk ( $50 \text{ ng g}^{-1}$ ), respectively. Black arrow indicate peak of sulfadimidine (I.S.= internal standard).



**Figure 2.** Plasma concentrations of sulfadimidine (Mean  $\pm$  SD) versus time after a single intravenous (i.v.) and oral (p.o.) administration (n=6).



**Figure 3.**  $C_{max}$ ,  $K_{10\_HL}$ ,  $T_{max}$  and  $Cl$  values of SDD in plasma after single oral administration of different doses (Mean  $\pm$  SD, n=6).

Table I. Pharmacokinetic characteristics of sulfadimidine in plasma after single intravenous (i.v.) and oral (p.o.) administration in laying hen (Mean  $\pm$  SD, n=6).

Dose	AUC	K01_HL	K10_HL	Cl	T <sub>max</sub>	C <sub>max</sub>
mg kg <sup>-1</sup>	μg h ml <sup>-1</sup>	h	h	ml h <sup>-1</sup> kg <sup>-1</sup>	h	μg ml <sup>-1</sup>
100 (i.v.)	1122.0 $\pm$ 245.8	-	5.9 $\pm$ 1.1	94.1 $\pm$ 27.8	-	132.2 $\pm$ 16.9
100 (p.o.)	1379.3 $\pm$ 428.1	1.2 $\pm$ 0.4	9.1 $\pm$ 1.6	82.5 $\pm$ 40.0	4.0 $\pm$ 0.9	76.5 $\pm$ 19.9
30 (p.o.)	151.5 $\pm$ 27.5	0.7 $\pm$ 0.6	2.8 $\pm$ 0.7	204.2 $\pm$ 41.6	1.5 $\pm$ 0.8	25.3 $\pm$ 5.8
10 (p.o.)	18.5 $\pm$ 4.1	0.4 $\pm$ 0.4	1.4 $\pm$ 0.8	563.2 $\pm$ 119.2	0.7 $\pm$ 0.5	6.2 $\pm$ 1.8
3 (p.o.)	4.2 $\pm$ 1.2	0.2 $\pm$ 0.3	0.9 $\pm$ 0.2	797.3 $\pm$ 337.1	0.5 $\pm$ 0.3	2.4 $\pm$ 1.1

AUC, area under the curve; K01\_HL, half life of absorption; K10\_HL, elimination half life; Cl, total body clearance; T<sub>max</sub>, time of maximum plasma concentration; C<sub>max</sub>, maximum plasma concentration.

Table II. Sulfadimidine residues in yolk ( $\mu\text{g g}^{-1}$ , Mean  $\pm$ SD) after single oral administration of different doses.

Dose ( $\text{mg kg}^{-1}$ BW)	100	30	10	3	1
Day after treatment					
1	14.32 $\pm$ 6.47*	3.08 $\pm$ 1.52*	0.42 $\pm$ 0.08	0.11 $\pm$ 0.05	ND
2	6.62 $\pm$ 2.72*	0.51 $\pm$ 0.01‡	0.07 $\pm$ 0.02	< 0.05	ND
3	3.67 $\pm$ 1.17*	0.44 $\pm$ 0.12*	0.06 $\pm$ 0.02*	ND	ND
4	2.74 $\pm$ 0.69*	0.32 $\pm$ 0.05†	< 0.05	ND	ND
5	1.98 $\pm$ 0.56*	0.20 $\pm$ 0.04‡	< 0.05	ND	ND
6	1.21 $\pm$ 0.59*	0.13 $\pm$ 0.02*	ND	ND	ND
7	0.43 $\pm$ 0.32*	0.07 $\pm$ 0.03‡	ND	ND	ND
8	0.12 $\pm$ 0.14‡	< 0.05	ND	ND	ND
9	< 0.05	ND	ND	ND	ND
10	ND	ND	ND	ND	ND

n=6 unless otherwise indicated while \*, † and ‡ = 5, 4, 3 eggs laid and analyzed, respectively. ND = not detected (< LOD)

Table III. Sulfadimidine residues in albumen ( $\mu\text{g g}^{-1}$ , Mean  $\pm$ SD) after single oral administration of different doses.

Dose ( $\text{mg kg}^{-1}$ BW)	100	30	10	3	1
Day after treatment					
1	$49.34 \pm 7.03^*$	$6.25 \pm 1.85^*$	$0.98 \pm 0.24$	$0.28 \pm 0.12$	$0.07 \pm 0.02$
2	$6.10 \pm 0.43^*$	$0.16 \pm 0.04^\ddagger$	ND	ND	ND
3	$0.83 \pm 0.16^*$	$0.15 \pm 0.14^*$	ND	ND	ND
4	$0.38 \pm 0.12^*$	ND	ND	ND	ND
5	$0.20 \pm 0.07^*$	ND	ND	ND	ND
6	$0.12 \pm 0.03^*$	ND	ND	ND	ND
7	< 0.05	ND	ND	ND	ND
8	ND	ND	ND	ND	ND

n=6 unless otherwise indicated while \*, †, ‡ = 5, 4, 3 eggs laid and analyzed, respectively. ND = not detected (< LOD)

TableIV. Transfer rate of SDD into eggs after single oral administration.

Dose (mg kg <sup>-1</sup> BW)	Drug intake <sup>a</sup> (mg)	Residue in whole egg <sup>b</sup> (µg)	Transfer-rate <sup>c</sup> (%)
100	195	2497.16	1.28
30	61.5	303.60	0.49
10	19.1	43.17	0.23
3	5.8	11.68	0.20
1	1.8	2.54	0.14

<sup>a</sup> calculated from dose × average BW, (data not shown)

<sup>b</sup> calculated according to equation [1]

<sup>c</sup> calculated according to equation [2]

Table V. Residue concentration of Sulfadimidine (Mean± SD) in plasma, albumen and yolk following medicated feed of different doses (30, 10 and 3 mg kg<sup>-1</sup> BW) for seven consecutive days.

Matrix Dose (mg kg <sup>-1</sup> BW)	Plasma (µg ml <sup>-1</sup> )			Albumen (µg g <sup>-1</sup> )			Yolk (µg g <sup>-1</sup> )		
	30	10	3	30	10	3	30	10	3
Day 1	4.87 ± 2.05	0.58 ± 0.17	0.11 ± 0.03	2.50 ± 1.10	0.42 ± 0.07	0.13 ± 0.03 <sup>‡</sup>	0.26 ± 0.14	<0.05	<0.05
Day 2	3.33 ± 1.97	0.52 ± 0.11	0.09 ± 0.03	5.40 ± 2.58 <sup>*</sup>	0.62 ± 0.15	0.15 ± 0.02	1.23 ± 0.52 <sup>*</sup>	0.10 ± 0.03	<0.05
Day 3	4.70 ± 1.88	0.47 ± 0.07	0.09 ± 0.02	4.86 ± 1.44	0.61 ± 0.15	0.15 ± 0.01	1.50 ± 0.70	0.13 ± 0.03 <sup>*</sup>	<0.05
Day 4	3.99 ± 1.52	0.44 ± 0.05	0.09 ± 0.03	4.99 ± 1.93 <sup>*</sup>	0.64 ± 0.05 <sup>*</sup>	0.14 ± 0.01	2.04 ± 0.60 <sup>*</sup>	0.15 ± 0.03	<0.05
Day 5	2.36 ± 2.41	0.43 ± 0.07	0.10 ± 0.03	3.77 ± 1.25 <sup>*</sup>	0.45 ± 0.06	0.13 ± 0.02 <sup>*</sup>	1.80 ± 0.52 <sup>*</sup>	0.16 ± 0.02	<0.05
Day 6	2.91 ± 1.08	0.31 ± 0.10	0.07 ± 0.01	2.84 ± 1.25 <sup>*</sup>	0.40 ± 0.12	0.14 ± 0.02	1.55 ± 0.45 <sup>*</sup>	0.20 ± 0.02	<0.05
Day 7	1.48 ± 0.95	0.37 ± 0.12	0.09 ± 0.02	2.97 ± 0.90 <sup>†</sup>	0.43 ± 0.14	0.13 ± 0.01	1.64 ± 0.55 <sup>†</sup>	0.20 ± 0.02	<0.05

<sup>\*</sup>, <sup>†</sup>, <sup>‡</sup> 5, 4 and 3 eggs laid and analyzed, respectively