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RESIDUE DEPLETION OF SULPHADIAZINE AND TRIMETHOPRIM IN PIGS AND BROILERS AFTER ORAL ADMINISTRATION

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ORIGINAL ARTICLE

ABSTRACT: *The residual behaviour of a sulphadiazine (SDZ) and trimethoprim (TMP) combination was studied in fourteen pigs and twenty-eight broilers. The drug combination was added in the amount of 700 mg kg^{-1} (SDZ) and 140 mg kg^{-1} (TMP) to pig and 300 mg kg^{-1} (SDZ) and 60 mg kg^{-1} (TMP) to broiler feed, respectively. The medicated feeds were supplied for 5 consecutive days. The tissue SDZ/TMP concentrations were measured by a HPLC method. To ensure safe residue levels in all target tissues, withdrawal time of 8.6 days and 6.0 days should be applied to pigs and broilers, respectively, treated with SDZ and TMP in feed.*

Key words: *Sulphadiazine; Trimethoprim; Pigs; Broilers; Residues; Withdrawal Time; Veterinary Drugs*

INTRODUCTION

Combinations of sulphadiazine (SDZ) and trimethoprim (TMP) are commonly used for the treatment of respiratory, gastrointestinal and urogenital infections in food producing animals. The large scale of application of this combination has led to the occasional occurrence of residues in edible tissues. These residue values could be particularly high presenting a hazard to human health if the recommended withdrawal times are not respected. The European Union allocated the two molecules in Table 1 of the Commission Regulation of the European Union (EU 2010). The MRLs fixed for pig and broiler tissues are 100 µg kg^{-1} and 50 µg kg^{-1} for SDZ and TMP, respectively.

For these two drugs, alone or in combination, several pharmacokinetic studies have been performed in pigs after intravenous (Nielsen and Rasmussen, 1975; Luther, 1979; Friis et al., 1984a,b; Gyrd-Hansen et al., 1984; Nouws et al., 1989) or oral administration (Søli et al., 1990; Nielsen and Gyrd-Hansen, 1994; Garwacki et al., 1996). The SDZ/TMP combination pharmacokinetic behaviour was also described after intramuscular injection or transdermal delivery (Sekido et al., 1992). Nevertheless, it is difficult to find information about the tissue distribution and tissue residue depletion of both compounds in this species. Similarly, notwithstanding their intensive use, very few published data are available on the pharmacokinetics and residual behaviour of these drugs in poultry (Loscher et al., 1990, Takahashi et al., 1991, Dagorn et al., 1992).

The aim of this study was to evaluate the residues of SDZ and TMP in pig and broiler edible tissues after oral administration of the two-drug combination in the feed under practical conditions. Based on the tissue residues, the withdrawal time of the combined drugs was calculated according to Guidelines of the Committee for Veterinary Medical Products of the European Agency for the Evaluation of Medicinal Products (EMEA, 1995).

MATERIALS AND METHODS

Animal treatment

Pigs: fourteen 60-day old pigs (Large White x Landrace) weighing 19.3±1 kg (mean body weight±SD) were obtained from a local farm. The animals were randomly allotted to 3 experimental groups (four pigs/group). Two pigs were used as controls. The pigs were housed in single boxes under controlled temperature and humidity. The animals were permitted *ad libitum* access to feed and water throughout the experiment. The feed and water consumption of each animal was determined every day. After 15 days of acclimatisation, SDZ and TMP were administered to pigs in the three experimental groups at 700 mg kg^{-1} and at 140 mg kg^{-1} of the diet, respectively. The medicated feed was supplied for 5 consecutive days. The time of slaughter was fixed at 1, 7 and 10 days after the end of the treatment. At each time point one group of animals was sacrificed and samples of the target tissues

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(muscle, liver, kidney and skin/fat) were taken and stored at –20 °C. At the last time point, also the control pigs were slaughtered.

Broilers: twenty-eight 45-day old broilers (Golden Comet) weighing 0.52±0.02 kg (mean body weight±SD) were obtained from a local farm. The animals were randomly allotted to 4 experimental groups (six broilers/group). Four broilers were used as controls. The broilers were caged individually under controlled temperature and humidity. The broilers were permitted *ad libitum* access to feed and water throughout the experiment. The feed and water consumption of each animal was determined every day. After 15 days of acclimatisation, SDZ and TMP were administered to the broilers in the four experimental groups at 300 mg kg^{-1} and at 60 mg kg^{-1} of the diet, respectively. The medicated feed was supplied for 5 consecutive days. The time of slaughter was predetermined at the 1, 3, 5 and 10 days after the end of the treatment. At each time point one group of animals was sacrificed and samples of target tissues (muscle, liver, kidney and skin/fat) were taken and stored at –20 °C. At the last time point, also the control broilers were slaughtered.

The study was carried out in observance of legislation concerning the use of animals for experimental purposes (D.L. 27/01/1992 no. 116).

Analytical procedures

Reagents: Sulphadiazine sodium salt and trimethoprim base were obtained from Sigma-Aldrich (Milan, Italy) and used to prepare the reference standard solutions. Methanol, acetonitrile and water, purchased from Mallinkrodt Baker (Deventer, the Netherlands), were of HPLC grade. Sodium chloride (NaCl), potassium phosphate (KH_2PO_4), sodium acetate (CH_3COONa), 85% orthophosphoric acid (H_3PO_4), 37% hydrochloric acid (HCl), purchased from Analyticals Carlo Erba (Milan, Italy), were of analytical grade. Clean-up cartridges (SPE-C18, 500 mg, 7020-06) were from J.T. Baker (Phillipsburgh, N.J. USA).

Solutions: a 0.02 M KH_2PO_4-buffer solution (pH 3) was prepared by dissolving KH_2PO_4 (2.72 g) in water (1 L); the pH was adjusted with H_3PO_4 (85%). A 0.025 M KH_2PO_4-buffer solution (pH 4.5) was prepared by dissolving KH_2PO_4 (3.40 g) in water (1 L); 500 mL of this buffer solution was adjusted to pH 3.5 by adding concentrated (85% w/v) H_3PO_4. 0.1 M HCl was prepared by diluting 0.83 mL of concentrated (37% w/v) HCl with 100 mL of H_2O. 0.5 M NaCl was prepared by dissolving 29.11 g L^{-1} and adjusting pH to 2.5 with 0.1 M HCl. 0.2 M CH_3COONa was prepared by dissolving 16.41 g L^{-1} in water. The HPLC mobile phase was made by mixing 0.02 M KH_2PO_4-buffer solution (pH 3) and CH_3CN at the ratio of 80:20 (v:v) and 83:17 (v:v) for SDZ and TMP analysis, respectively.

Standard solutions: stock standard solutions of the two drugs (200 µg mL^{-1}) were prepared separately by dissolving 10 mg of SDZ with 50 mL of methanol and 10 mg of TMP with 10 mL of methanol subsequently diluted to 50 mL with water. Both stock standard solutions were stored at –20±1 °C; under these conditions their stability is 1 month. The working standard solutions were made by diluting aliquots of the stock solutions in a 0.02 M KH_2PO_4-buffer (pH 3) to obtain concentrations ranging from 0.05 to 2 µg mL^{-1} for SDZ and from 0.02 to 1 µg mL^{-1} for TMP. Fortification solutions, containing SDZ at 0.5, 1, 5, 10 or 20 µg mL^{-1} and TMP at 0.2, 0.5, 1, 5 or 10 µg mL^{-1}, were prepared in water from stock solutions. Fortification was carried out by adding 50 µL (for SDZ) and 40 µL (for TMP) of these solutions to 1 g of the homogenised tissues.

Sample preparation and clean up: target tissues were cut with scissors to obtain small pieces and 1±0.1 g of tissue was weighted. To this amount of sample, was added water (1 mL) and methanol (3 mL), and was homogenised with an Ultraturrax (IKA Labortechink). The following steps differed for the SDZ and TMP assay.

Sulphadiazine: the homogenate was centrifuged (15 min) at 1000 x ***g*** (Beckman GPK). The supernatant was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 20 mL of 0.5 M NaCl (pH 2.5) and applied to a SPE-C18 cartridge prewashed with methanol (2 mL), water (2 mL) and 0.5 M NaCl (pH 2.5) (2 mL). The cartridge was washed with 1 mL of 0.5 M NaCl (pH 2.5) and SDZ was eluted with 2 mL of methanol:water (1:1 v:v). The eluate was evaporated to dryness under a stream of nitrogen, then redissolved with 500 µL of methanol:water (1:1 v:v) and transferred into vials for HPLC analysis.

Trimethoprim: the homogenate was diluted with 0.2 M CH_3COONa (20 mL) and then centrifuged at 1000 x ***g*** (15 min). The supernatant was cleared on SPE-C18 cartridge prewashed with CH_3OH (2 mL) and H_2O (2 mL). After the sample loading, the cartridge was washed with a 0.025 M KH_2PO_4-buffer solution (pH 4.5) (2 mL) and TMP was eluted with 2 mL of a mixture 0.025 M KH_2PO_4-buffer solution (pH 3.5):CH_3OH (10:90 v:v). The eluate was dried under vacuum (UNIVAPO, A.N. Kraupa), dissolved in 400 µL of 0.025 M KH_2PO_4-buffer solution (pH 4.5) and transferred into vials for HPLC analysis.

High-performance liquid chromatography

The chromatographic analyses of SDZ and TMP were performed by an HPLC system (Beckman System Gold equipped with UV-Diode array Beckman 168 detector and GOLD release 4.0 software (Beckman Inst. INC) and a reverse phase column ABZ (5 µm; 250x4.6 mm - Supelco) under the following conditions: mobile phases: see

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above; flow rate: 0.6 mL min^{-1}; injection volume: 50 µL; detection wavelength: 272 nm for SDZ and 230 nm for TMP. The total run time was 15 min.

Method validation

Linearity of the detector response was checked with the standard solution; the range was 0.05 to 2.0 µg mL^{-1} for SDZ and 0.02 to 1.0 µg mL^{-1} for TMP. Selectivity was evaluated by comparing the chromatograms of blank and spiked samples processed under the described conditions. Accuracy of the analytical method was assessed for SDZ and TMP by replicate analyses of samples fortified at 0.1-0.5-1 µg/g and was reported as percent recovery (rec%).

The inter-day precision of the method was checked, for each different tissue, by repetitively analysing tissues samples spiked at 0.1 µg/g for both the drugs and was expressed as coefficient of variation % (CV%).

The detection limit (LOD) was estimated by visual evaluation of the minimum level at which the two analytes can be reliably detected. The quantification limit (LOQ) was determined by the analyses of samples with known concentrations of analytes and by establishing the minimum level at which the single analytes could be quantified with accuracy and precision that fall within the range recommended by the EMEA (1996, 1998).

Calculation of withdrawal times

As suggested by the Committee for Veterinary Medicinal Products (EMEA 1995), withdrawal periods were set at the time point at which the concentrations of residues in all tissues for all animals fall below the respective MRL values. In order to compensate for the uncertainties of biological variability, the estimation of a safety span (10-30% of time period) was considered. A statistical model based on linear regression analysis was also used as an alternative approach to estimate withdrawal periods.

All residues of SDZ and TMP, which were below the LOQ as well as the MRL, were calculated and reported exactly. When residue values were also below the LOD, they were entered at half of the LOD value for calculation purposes. When all the data at a particular time point were lower than the LOD, the results were excluded from calculation. Regression models were fitted to the logarithms of the muscle, liver, kidney and skin/fat SDZ and TMP residue concentrations. Non-linearity of each regression model was assessed using the lack of fit test. Homogeneity of variances was assessed using Cochran and Barlett's methods, and the normality of residuals was checked using the Shapiro-Wilk test. One-sided upper tolerance limits (95%) with a 95% confidence were calculated from these regression models, based on the equation of Stange.

RESULTS AND DISCUSSION

The finalised analytical conditions gave retention times of 8.5±0.1 min and 7.2±0.1 min for SDZ and TMP, respectively. At these retention times, no interfering peaks were observed in the blank samples of the matrices examined. Representative HPLC chromatograms for sulphadiazine are reported in Figure 1 and for trimethoprim in Figure 2.

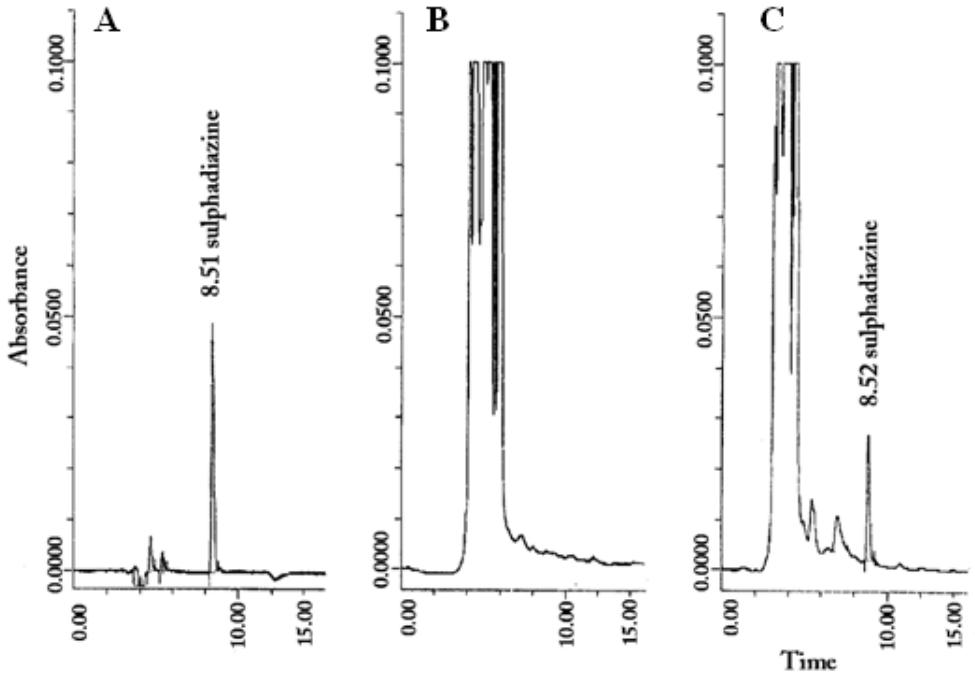

Figure 1. Representative HPLC chromatograms of (A) TMP standard (500 ng ml^{-1}), (B) a blank extracted broiler kidney sample and (C) an extracted broiler kidney sampled after treatment.

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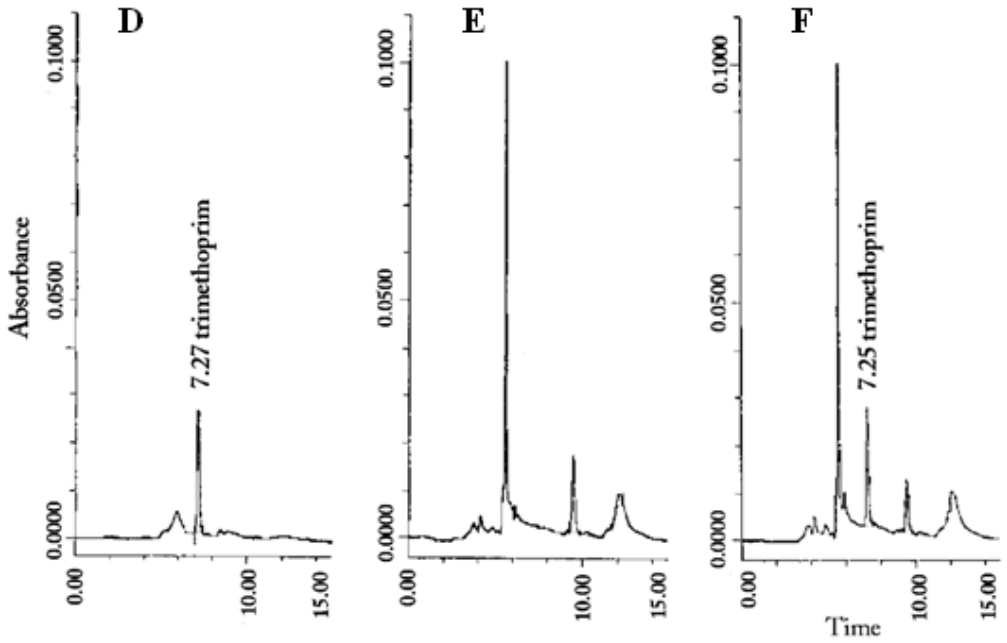

Figure 2. Representative HPLC chromatograms of (D) SDZ standard (500 ng ml^{-1}), (E) a blank extracted broiler kidney sample and (F) an extracted broiler kidney sampled after treatment.

The calibration curves for the two test antibacterial drugs were linear over the concentration ranges examined (i.e. SDZ 0.05-2.0 µg mL^{-1}; TMP 0.02-1.0 µg mL^{-1}) with correlation coefficients always greater than 0.999. The average recoveries (±SD) determined for each tissue and for both drugs at three different concentration levels, are reported in Table 1. Recoveries in the target tissues ranged from 76.79±0.74% (broiler) and 78.82±1.07% (pig) for SDZ and between 77.02±0.62% (broiler) and 80.49±1.15% (broiler) for TMP. The precision data expressed as CV%-values are given in Table 2.

Table 1 - The mean recovery (%), SD and CV (%) of sulphadiazine and trimethoprim from different spiked samples (n=6)

PIG									BROILER								
	Sulphadiazine				*Trimethoprim*					*Sulphadiazine*				*Trimethoprim*			
Tissue	mean	±	SD	CV%	mean	±	SD	CV%		mean	±	SD	CV%	mean	±	SD	CV%
Muscle	77.84	±	0.89	1.15	78.09	±	1.00	1.28	*muscle*	77.20	±	0.91	1.18	78.91	±	1.18	1.50
Liver	78.11	±	0.72	0.92	78.51	±	1.01	1.29	*liver*	77.84	±	1.11	1.43	78.22	±	1.36	1.74
Kidney	77.07	±	0.92	1.20	77.22	±	1.00	1.33	*kidney*	76.88	±	0.65	0.85	80.49	±	1.15	1.43
Fat	78.82	±	1.07	1.35	79.96	±	0.82	1.04	*fat*	76.79	±	0.74	0.97	77.02	±	0.62	0.80

Table 2 - Precision of sulphadiazine and trimethoprim determination at 0.1 µg g^{-1} in pig and broiler tissues (mean value and CV%; n=6)

PIG					BROILER				
	Sulphadiazine		*Trimethoprim*			*Sulphadiazine*		*Trimethoprim*	
Tissue	mean	CV%	mean	CV%	Tissue	mean	CV%	mean	CV%
Muscle	0.073	2.29	0.076	2.70	*Muscle*	0.075	3.44	0.076	1.35
Liver	0.073	1.12	0.076	1.97	*Liver*	0.075	3.60	0.075	3.37
Kidney	0.071	2.22	0.074	2.88	*Kidney*	0.085	3.17	0.078	2.41
Fat	0.073	2.83	0.075	2.16	*Fat*	0.083	1.91	0.077	1.44

In pigs, the inter-day precision ranged from 1.12% (liver) to 2.83% (skin/fat) for SDZ and from 1.97% (liver) to 2.88% (kidney) for TMP. In broilers, the inter-day precision ranged from 1.91% (skin/fat) to 3.44% (muscle) for SDZ and from 1.35% (muscle) to 3.37% (liver) for TMP. The LOD was defined for all tissues at 0.025 µg mL^{-1} for SDZ and at 0.020 µg mL^{-1} for TMP. For both drugs, a single LOQ value were validated for all tissues of the two animal species corresponding to one-half the MRL values (0.05 µg g^{-1} for SDZ and 0.025 µg g^{-1} for TMP). The mean values (±SD) of SDZ and TMP residues in target tissue of treated pigs and broilers are reported in Table 3 and Table 4, respectively.

One day after the intake of the last dose, SDZ and TMP tissue levels in pigs were higher than the corresponding MRLs in muscle, liver and kidney. In contrast, in skin/fat, while the SDZ residues were lower than the reference values (MRLs and LOQ), those for TMP were all higher than the defined residual limits.

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Table 3 - The mean (±SD) concentrations (µg g^{-1}) of sulphadiazine and trimethoprim in pig (4/group) tissues after oral administration

PIG						
Time after treatment (days)						
	Sulphadiazine			*Trimethoprim*		
Tissue	1	7	10	1	7	10
Muscle	0.172±0.015	0.068±0.018	-	0.099±0.015	0.036±0.004	-
Liver	0.234±0.031	0.063±0.006	-	0.171±0.010	0.035±0.006	-
Kidney	0.282±0.018	0.056±0,008	-	0.306±0.029	0.038±0.002	-
Fat	-	-	-	0.134±0.019	-	-

Table 4 - The mean (±SD) concentrations (µg g^{-1}) of sulphadiazine and trimethoprim in broiler (6/group) tissues after oral administration

BROILER								
Time after treatment (days)								
	Sulphadiazine				*Trimethoprim*			
Tissue	1	3	5	10	1	3	5	10
Muscle	0.102±0.011	-	-	-	0.038±0.006	-	-	-
Liver	0.187±0.054	0.081±0.006	0.058±0.002	-	0.066±0.017	-	-	-
Kidney	0.369±0.064	0.154±0.056	0.065±0.004	-	0.249±0.039	0.082±0.006	0.033±0.006	-
Fat	0.447±0.057	0.217±0.077	0.076±0.010	-	0.225±0.035	0.079±0.011	0.038±0.005	-

Sulphadiazine concentrations decreased rapidly during the following six days and reached levels lower than the MRLs in all target tissues. Nevertheless, it was detected over 0.05 µg g^{-1} (LOQ) in 3 muscles, 2 livers and 2 kidneys. Similarly, at the same time point, residual values of TMP were always lower than the MRLs, but higher than the LOQ in 2 muscle, 3 liver and 3 kidney samples. In skin/fat, TMP residues were all lower than the LOQ, on day 7.

Ten days after the end of the treatment both SDZ and TMP concentrations detected were lower than the LOQ in all tissues for all animals.

One day after the end of the treatment, SDZ residues in broilers were detected at values below the MRLs in only muscle from three animals. In all the other tissue samples, drug concentrations were higher than 100 µg g^{-1}. On the subsequent sampling time at day 3, the SDZ concentrations in all muscles were below the LOQ and in all livers below the MRLs. In contrast, the mean concentrations detected in kidney and skin/fat were still fairly high (0.154±0.06 µg g^{-1} and 0.217±0.08 µg g^{-1}). In these two last tissues, the SDZ residues did not fall below the MRLs until day 5 after withdrawal of treatment. At the following time point residual levels decreased also under the LOQ in all the analysed samples. At the first sampling point, TMP concentrations in broilers were lower than the LOQ in muscle from 4 animals and lower than the MRLs in the other two muscles and in one liver. The observed values in kidney and skin/fat were always over 0.050 µg g^{-1}.

After two days, drug concentrations decreased below 0.025 µg g^{-1} in all the muscle and in all the liver samples. TMP depletion in the other two tissues was slower and concentrations were below the validated LOQ in all tissues for all animals only at the last sampling point.

Sulphadiazine withdrawal periods calculated for muscle, liver, kidney and skin/fat were 8.4, 8.4, 8.4, 1.0 days and 3.0, 3.6, 6.0, 6.0 days in pig and broiler tissues, respectively. TMP withdrawal periods established for muscle, liver, kidney and skin/fat were 8.4, 8.4, 8.4, 7.0 days and 1, 3.6, 6.0, 6.0 days in pigs and broilers, respectively.

The analytical methods adopted in this study to evaluate the residual concentrations of SDZ and TMP in pig and broiler edible tissues had shown good selectivity, sensitivity and percentage of recovery from spiked tissues. The extraction and purification procedures of the two drugs from tissues appeared simple and show a good repeatability. The samples were processed in a short time and without hazardous wastes. The limits of quantitation (LOQ) appeared suitable for the residue depletion studies. The experimental protocol for the residue depletion study simulated the field conditions. The variation of the feed consumption and, as a consequence, of the oral dose of sulfadiazine and TMP during the treatment was within acceptable limits (mean CV: 4.25% for pigs and 8.21% for broilers, respectively).

The use of the statistical linear regression model to estimate the withdrawal times requires that some regression assumptions such as homogeneity of variances of the $\log_e$-transformed data on each slaughter day, linearity of the $\log_e$-transformed data versus time and a normal distribution of the errors are valid. Our residual data detected in pig and broiler tissues did not always satisfy one or more of these conditions (Table 5). For this reason, the withdrawal periods established correspond to the time points at which the concentration of residues in all tissues for all animals fell below the respective MRLs plus a 20% safety span. When all observations were below the LOQ, this safety span value was not applied. Garwacki et al. (1996) following administration to pigs of a medicated SDZ/TMP feed (30 mg kg^{-1} bw/6 mg kg^{-1}bw) for five days, found that both drugs were rapidly eliminated. Five days after the treatment, SDZ was not detected in any tissue, whereas TMP was present at concentrations of 0.01 µg g^{-1} (muscle), 0.02 µg g^{-1} (liver) and 0.03 µg g^{-1} (kidney).

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Table 5 - Results of statistical linear regression model applied for the withdrawal period evaluation in pigs and broilers after oral administration of sulphadiazine and thrimetoprim

Sulphadiazine						
		F-test	**Cochran-test**	**Barlett-test**	**Shapiro/Wilk**	**WT**
	Tissue	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.10)	(days)
Pig	Muscle	p>0.05	0.01<p<0.05	manually performed[2]	p>0.10	-
	Liver	0.05>p>0.025	p>0.05	manually performed	0.05>p>0.02	-
	Kidney	p<0.025	p>0.05	manually performed	0.10>p>0.05	-
	Fat[1]	-	-	-	-	-
Broiler	Muscle	p<0.025	p>0.05	p<0.01	p>0.1	-
	Liver	p>0.05	p>0.05	p>0.05	p>0.1	5.3
	Kidney	p>0.05	p<0.01	p>0.1	p>0.1	-
	Fat	p>0.05	p<0.01	0.05>p>0.025	p>0.1	-
Thrimetoprim						
		F-test	**Cochran-test**	**Barlett-test**	**Shapiro/Wilk**	**WT**
	Tissue	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.10)	(days)
Pig	Muscle	p>0.05	p>0.05	manually performed[2]	p>0.10	8.3
	Liver	p>0.05	p>0.05	manually performed	p>0.10	7.9
	Kidney	p>0.05	p>0.05	manually performed	p>0.10	8.6
	Fat[1]	p>0.05	p<0.01	manually performed	p<0.01	-
Broiler	Muscle	-	-	-	-	-
	Liver	p>0.05	p<0.01	p<0.01	0.10>p>0.05	-
	Kidney	p>0.05	p<0.01	p<0.01	p<0.01	-
	Fat	p>0.05	p>0.05	p>0.05	p>0.10	-

[1]not statistically analyzed because residual concentrations were below the corresponding MRL and LOQ at the first time point. [2]because each group number <5

Eight days after the last dose the drug was not detected in any tissue. On the basis of their results, the authors proposed a withdrawal time not less than 5 days for the formulation used in pigs. In contrast with these findings, one day after the last intake, we found levels of SDZ higher than those of TMP in the muscle and liver and lower in the kidney. Sulphadiazine was detected until 7 days after the last intake. In addition, TMP residual depletion was more prolonged and values detected at this time point were close to the MRL. These differences could be related to physiological or environmental conditions, particularly to the free access to feed, with a consequent different daily intake of the two drugs during the present residual depletion studies. Dagorn et al. (1992), after a SDZ/TMP combination administered at 20/4 mg kg^{-1} b.w./daily in broilers via drinking water for 4 days, observed a more rapid decrease of TMP. Forty-nine hours after the end of the medication, TMP residue concentrations were detected only in skin with values close to 0.05 µg g^{-1}, whereas SDZ skin concentrations reached mean values of 0.14±0.04 µg g^{-1}. The calculated withdrawal time was 7.28 days. In contrast, in our study the SDZ withdrawal period gave evidence of a similar rapid decrease of this drug, notwithstanding different levels detected on the first time point in the liver, kidney and skin/fat.

CONCLUSION

To ensure safe residue levels in all target tissues, withdrawal periods of 8.6 days and 6.0 days should be applied to pigs and broilers treated with 700 ppm of SDZ and 140 ppm of TMP and with 300 ppm of SDZ and 60 ppm of TMP in feed, respectively.

REFERENCES

European Medicines Agency (EMEA) (1995). Approach Towards Harmonisation of Withdrawal Periods (EMEA/CVMP/036/95). European Agency for the Evaluation of Medicinal Products, Committee for Veterinary Medical Products. Available from: http://www.ema.europa.eu/pdfs/vet/swp/003695en.pdf.

European Medicines Agency (EMEA) (1996). Position Paper on Requirements for LOQ/MRL ratio (EMEA/CVMP/274/96-FINAL). European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit. Available from: http://www.eudra.org/emea.html

European Medicines Agency (EMEA) (1998). Guideline on validation of analytical procedures: methodology (EMEA/CVMP/591/98-FINAL). European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004340.pdf

Europea Union (EU) (2010). Commission Regulation N. 37/2010 of 22 December 2009 on Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of the European Union 20.1.2010.

Dagorn M, Laurentie M, Delmas JM, Guillot P and Sanders P (1992). Tissue residues of trimethoprim (TMP) and sulfadiazine (SDZ) combination administered to broiler. In Proceedings of the 3rd World Congress on Foodborne Infections and Intoxications, Berlin, Germany, 16-19 June, 2: 1276-1278.

Friis C, Gyrd-Hansen N, Nielsen P, Olsen CE and Rasmussen F (1984a). Pharmacokinetics and metabolism of sulphadiazine in neonatal and young pigs. Acta Pharmacologica et Toxicologica, 54: 321-326.
Friis C, Gyrd-Hansen N, Nielsen P, Nordholm L and Rasmussen F (1984b). Pharmacokinetics and metabolism of trimethoprim in neonatal and young pigs. Pediatric Pharmacology, 4: 231-238.
Garwacki S, Lewicki J, Wiechetek M, Grys S, Rutkowski J and Zaremba M (1996). A study of the pharmacokinetics and tissue residues of an oral trimethoprim/sulphadiazine formulation in healthy pigs. Journal Veterinary Pharmacology and Therapeutics, 19: 423-430.
Gyrd-Hansen N, Friis C, Nielsen P and Rasmussen F (1984). Metabolism of trimethoprim in neonatal and young pigs: comparative *in vivo* end *in vitro* studies. Acta Pharmacologica et Toxicologica, 55: 402-409.
Loscher W, Fassbender CP, Weissing M and Kietzmann M (1990). Drug plasma levels following administration of trimethoprim and sulphonamide combinations to broilers. Journal Veterinary Pharmacology and Therapeutics, 13: 309-319.
Luther HG (1979). The pharmacokinetics of sulphadiazine in cattle, sheep and swine. Dissertation Abstracts International B, 39: 5789-5790.
Nielsen P and Rasmussen F (1975). Half-life and renal excretion of trimethoprim in swine. Acta Pharmacologica et Toxicologica, 36: 123-131.
Nielsen P and Gyrd-Hansen N (1994). Oral bioavailability of sulphadiazine and trimethoprim in fed and fasted pigs. Research in Veterinary Science, 56: 48-52.
Nouws JFM, Mevius D, Vree TB and Degen M (1989). Pharmacokinetics and renal clearance of sulphadimidine, sulphamerazine and sulphadiazine and their N4-Acetyl and hydroxy metabolites in pigs. Veterinary Quarterly, 11: 78-86.
Sekido SE, Schwark WS and Guard CL (1992). Transdermal delivery and intramuscular injection of trimethoprim/ sulphadiazine in sucking piglets. Veterinary Quarterly, 14: 85-87.
Søli NE, Framstad E, Skjerve S Sohlberg and Ødegaard SA (1990). A comparison of some of the pharmacokinetic parameters of three commercial sulphadiazine/trimethoprim combined preparation given orally to pigs. Veterinary Research Communication, 14: 403-410.
Takahashi Y, Said AA, Hashizume M and Kido Y (1991). Sulphadimethoxine residue in broiler-chicken skin. Journal of Veterinary Medical Science, 53: 33-36.

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