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Research paper

Tissue distribution of sulphadimidine sodium in non-starved and starved grower turkeys (*Meleagris gallopavo*)

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Abstract

Background: The use of veterinary drugs in food-producing animals has potential to generate residues in edible tissues and posses health hazard to consumers especially when the withdrawal period is not observed.
Objectives: The study was conducted to determine the tissue residue and withdrawal period of sulphadimidine in non-starved and starved grower turkeys following a single intramuscular administration.
Methods: Forty two turkeys of both sexes and 12 weeks old weighing 1.57±0.2 kg were divided into two groups of twenty one each. One group was administered a single intramuscular dose of sulphadimidine sodium (100mg/kg body weight). The other group was kept off-feed for 48 hours before drug administration. Three turkeys each were sacrificed from the starved and non-starved group and two grammes (2g) of tissue sample were harvested from selected tissues.
Results: The results showed that the drug residues persisted in all the tissues of turkeys sampled for up to thirty (30) days after drug administration. The starved turkeys maintained consistently higher concentrations of the drug in the tissues than fed ones. Sulphadimidine residue was significantly increased ($p<0.05$) between days 3 to 6 in the spleen of non-starved turkeys. However, the concentrations in the spleen decreased significantly ($p<0.05$) between days 6 to 10 and 25 to 30.
Conclusion: Sulphadimidine residue persisted in the tissues of non-starved and starved turkeys for up to 30 days after intramuscular injection. This should be given due consideration in the estimation of the withdrawal period for the drug, since sulphadimidine residue in meat > 0.2 ppm is unsafe for human consumption.

Keywords: *Maximum Residue Limit; Sulphadimidine; Grower Turkeys; Hypersensitivity*.

1. Introduction

Starvation responses are defined by physiological changes, such as rate of mass loss or nitrogen excretion or by the primary physiological fuel (lipid, carbohydrate or protein) used during starvation (Hervant & Renault, 2002, Caloin, 2004). Many acidic drugs including sulphadimidine bind to plasma albumin which is a component of proteins that are considered to be a biological fuel of last result during starvation (Baggot, 2001, Caloin, 2004).
Residues of veterinary medicinal products, as defined by the European Union, are pharmacologically active substances (whether active principles, excipients or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the veterinary medicinal product in question has been administered (Council Regulation, 1990).
The maximum residue level (MRL) of sulphonamides in poultry tissues and eggs is 100 µg/kg (FAO/WHO, 1992, Codex Alimentarius, 1996). The purpose of the MRL is to limit the exposure of consumers to residues of medicines used in food animals, to concentrations that do not pose human health risk (Kennedy *et al.*, 2000).
Poultry meat and eggs are very commonly consumed by humans. However, there may be situations where contaminations of drug residues in poultry products occur. Veterinary drugs and feed additives, especially anticoccidials and antibacterials e.g. sulphonamides are drugs most commonly used on poultry farms. They can be easily absorbed and distributed through the body of chickens, accumulated in various tissues and transferred into their products (Kan & Petz, 2000, Weiss *et al.*, 2007).
A number of possible adverse health effects of veterinary drug residues have been suggested which include the following; allergic/toxic reactions to residues, chronic toxic effects occurring with prolonged exposure to low levels of antibiotics and development of antibiotic-resistant bacteria in treated animals. These bacteria might then cause difficult-to-treat human infections or/and disruption of normal human flora in the intestine (Doyle, 2006).
Tissue distribution of sulphadimidine have been reported in broilers (Onyeyili *et al.*, 2000), laying hen (Nouws *et al.*, 1988), guinea fowl, domestic chicken and duck (Onyeyili *et al.*, 1997) and turkey poults (Heath *et al.*, 1975). In view of this, there is need to assay sulphadimidine sodium (100mg/kg) administered via the intramuscular route in non-starved and starved turkeys with a view to determining the withdrawal period in grower turkeys.

2. Materials and methods

2.1. Experimental animals and design

Forty two (42) turkeys of both sexes and 12 weeks old were divided into two groups of twenty one each. Two separate turkeys were first sacrificed and used for the preparation of tissue standards making a total of 44 turkeys. The turkeys were purchased from

commercial turkey breeders in Ibadan, Nigeria. The first group was administered sulphadimidine (non-starved) and the second group was starved for 48 hours before administration of sulphadimidine. All the animals were raised on deep litter system and stabilized for two weeks prior to experimentation. They were fed growers mash® and water was provided ad libitum. The animals were handled according to the international guilding principle for biomedical research involving animals (CIOM & ICLAS, 2012) and approved by the Ethical Committee, College of Veterinary Medicine, University of Agriculture Makurdi, Nigeria.

2.2. Drug administration and sampling

Sulphadimidine sodium (33.3%) produced by Kepro, Holland was used for the study at a dose of 100mg/kg body weight. All the turkeys were administered sulphadimidine sodium on the right pectoral muscle. Three turkeys each were sacrificed from the starved and non-starved group on days 3, 6, 10, 15, 20, 25 and 30 post drug administrations. Two grammes (2g) of tissue samples were harvested from the liver, kidney, spleen, heart, lung and left pectoral muscle.

2.3. Assay of tissue sulphadimidine

Free sulphadimidine in serum was determined using the method of Bratton and Marshall (1939) and revised by Salinas *et al.,* 1990). For the analysis of sulphadimidine in tissues, 0.2g of each tissue sample was crushed into fine particles and mixed with 3.80ml of distilled water and treated with 1ml of 20% trichloroacetic acid. After thorough mixing, the samples were allowed to stand for 10mins and cooled at -40°C. They were centrifuged at 3000 rpm for 10mins. To 2ml of clear supernatant, 0.1ml of 0.1% sodium nitrate was added and mixed. The mixtures were allowed to stand for 3mins followed by addition of 0.2ml of 0.5% ammonium sulphamate and mixed. The samples were allowed to stand for 2 mins before adding 0.2ml of 0.5% N-(1-naphthyl) ethylene diammine dihydrochloride. The samples were mixed and the optical density of the resulting color determined at 540nm wavelength using spectrophotometer (spectrum lab 23A, 340-1000nm). The limit of detection (LOD) of the assay was 0.05 µg/ml. The linear calibration curve of sulphadimidine in the tissues, within the range of 1-5µg/ml was obtained by plotting percentage absorbance against drug concentration. The correlation coefficient (R^2) was 0.93 for spleen, 0.95 for cardiac muscle, 0.90 for kidney, 0.94 for lungs, 0.88 for pectoral muscle and 0.98 for liver. The concentration of sulphadimidine in tissues was calculated using the formula below:

Concentration of Drug

$$= \frac{\text{Concentration of Standard x Optical Density of drug}}{\text{Optical Density of Standard}}$$

2.4. Pharmacokinetics analysis

Tissue elimination half-life ($T_{1/2\beta}$) and elimination rate constant (β) were calculated using established equations (Baggot, 2001).

2.5. Statistical analysis

Tissue concentrations, half-life and elimination rate constant of sulphadimidine were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test for individual comparisons. The significance level was set at $p<0.05$.

3. Results

Mean liver concentration of sulphadimidine residue of 9.16±0.86 ppm was obtained in non-starved turkeys while 9.51±0.60 ppm of sulphadimidine residue was obtained in starved turkey on day 3. The concentration decreased significantly ($p<0.05$) between day 25 to 30 in the liver of non-starved turkeys. However, in the starved turkeys, the concentrations increased significantly ($p<0.05$) between day 20 to 25 and decreased significantly ($P<0.05$) between day 25 to 30 (Table 1).

Table 1: Concentration of Sulphadimidine Residues (Ppm) in Liver of Non-Starved and Starved Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Time (Days)	Non-starved turkeys (ppm)	Starved turkeys (ppm)
3	9.16±0.86	9.51±0.60
6	9.76±0.26	10.25±0.31
10	10.00±0.86	10.85±0.39
15	10.50±2.96	11.54±0.47
20	14.76±0.88	12.08 ± 0.74^{c}
25	5.79 ± 1.86^{a}	15.30 ± 0.82^{d}
30	5.79 ± 1.86^{b}	6.53 ± 1.94^{de}

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. Superscript written as alphabets indicate significant difference ($P<0.05$).

Mean kidney concentration of sulphadimidine residue of 5.95±0.51 ppm was obtained in non-starved turkeys while 5.82±0.10 ppm of sulphadimidine residue was obtained in starved turkey on day 3. These concentrations increased until a peak concentration of 7.32±0.65 ppm and 8.69±1.84 ppm was obtained on day 20 in non-starved and starved turkeys respectively. The peak kidney concentration subsequently decreased and on day 30 post sulphadimidine administration, the kidney residue concentration was 2.87±1.70 ppm and 4.38±1.05ppm in non-starved and starved turkeys respectively (Table 2). There was no significant difference ($p>0.05$) between the kidney of non-starved and starved turkeys.

Table 2: Concentration of Sulphadimidine (Ppm) in Kidney of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Time (Days)	Non-starved turkeys (ppm)	Starved turkeys (ppm)
3	5.95±0.51	5.82±0.10
6	5.55±0.51	4.51±0.69
10	5.36±0.91	5.36±0.91
15	7.00±3.25	5.95±2.06
20	7.32±0.65	8.69±1.84
25	4.64±1.68	5.95±0.07
30	2.87±1.70	4.38±1.05

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. ($P>0.05$) within and between the groups.

Mean pectoral muscle concentration of sulphadimidine residue of 10.95±3.59 ppm was obtained in non-starved turkeys while 8.96±5.18 ppm of sulphadimidine residue was obtained in starved turkey on day 3. A peak concentration of 14.92±5.25 ppm was obtained in non-starved turkeys on day 10 while in starved turkeys, a peak concentration of 20.40±4.91 ppm was obtained on day 6. The peak kidney concentration subsequently decreased and on day 30 post sulphadimidine administration, the pectoral muscle concentrations were 0.99±0.50 ppm and 1.49±0.86 ppm in non-starved and starved turkeys respectively (Table 3). There was no significant difference ($p>0.05$) between the pectoral muscle of non-starved and starved turkeys.

Table 3: Concentration of Sulphadimidine (Ppm) in Pectoral Muscle of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Time (Days)	Non-starved turkeys (ppm)	Starved turkeys (ppm)
3	10.95±3.59	8.96±5.18
6	13.93±2.17	20.40±4.91
10	14.92±5.25	14.92±8.76
15	9.95±1.32	11.94±4.80
20	10.45±5.66	13.93±1.80
25	3.48±1.32	5.97±1.73
30	0.99±0.50	1.49±0.86

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for

windows followed by Bonferroni post test. (P>0.05) within and between the groups.
Sulphadimidine residue was significantly increased (p<0.05) between days 3 to 6 in the spleen of non-starved turkeys. However, the concentrations in the spleen decreased significantly (p<0.05) between days 6 to 10 and 25 to 30 (Table 4).

Table 4: Concentration of Sulphadimidine (Ppm) in Spleen of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Time (Days)	Non-starved turkeys (ppm)	Starved turkeys (ppm)
3	3.10±0.28^{f}	3.17±0.22
6	5.38±1.31^{g}	6.58±1.00
10	4.06±0.41^{gh}	4.10±0.28
15	2.94±0.37	2.75±0.22
20	2.94±0.44	2.63±0.32
25	1.86±0.24^{i}	3.37±0.27
30	0.54±0.37^{j}	0.58±0.58

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. Superscript written as alphabets indicate significant difference (P<0.05).

Mean lung concentration of sulphadimidine residue of 2.80±1.68 ppm was obtained in non-starved turkeys while 3.08±1.54 ppm of sulphadimidine residue was obtained in starved turkey on day 3. A peak concentration of 3.09±0.82 ppm and 3.38±2.41 ppm was obtained on day 15 in non-starved and starved turkeys respectively. The peak lung concentration subsequently decreased and on day 30 post sulphadimidine administration, the lung concentration was 0.29±0.00 ppm and 0.48±0.10 ppm in non-starved and starved turkeys respectively (Table 5).

Table 5: Concentration of Sulphadimidine (Ppm) in Lungs of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Time (Days)	Non-starved turkeys (ppm)	Starved turkeys (ppm)
3	2.80±1.68	3.08±1.54
6	1.45±0.93	1.64±1.36
10	1.74±0.29	3.09±0.38
15	3.09±0.82	3.38±2.41
20	1.25±0.82	2.80±1.60
25	0.29±0.17	1.45±0.67
30	0.29±0.00	0.48±0.10

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. (P>0.05) within and between the groups.

There was no significant difference (p>0.05) between the lung of non-starved and starved turkeys.
Mean cardiac muscle concentration of sulphadimidine residue of 1.27±0.84 ppm was obtained in non-starved turkeys while 1.91±0.83 ppm of sulphadimidine residue was obtained in starved turkey on day 3. These concentrations increased until a peak concentration of 4.46±2.07 ppm was obtained on day 20 in non-starved turkeys while in starved turkeys a peak concentration of 4.30±1.81 ppm was obtained on day10. The peak cardiac muscle concentration subsequently decreased and on day 30 post sulphadimidine administration, the cardiac muscle concentration was 1.91±1.68 ppm and 2.55±1.31ppm in non-starved and starved turkeys respectively (Table 6). There was no significant difference (p>0.05) between the cardiac muscle of non-starved and starved turkeys.

Table 6: Concentration of Sulphadimidine (Ppm) in Cardiac Muscle of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Time (Days)	Non starved turkeys (ppm)	Starved turkeys (ppm)
3	1.27±0.84	1.91±0.83
6	2.07±1.84	3.18±1.52
10	2.71±1.84	4.30±1.81
15	3.03±2.57	2.55±1.39
20	4.46±2.07	3.03±0.89
25	3.34±1.93	3.83±1.73
30	1.91±1.68	2.55±1.31

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. (P>0.05) within and between the groups.

The half-life of sulphadimidine in the various tissues was not significantly different (P>0.05) between starved and non-starved domestic grower turkeys. However the half-life of sulphadimidine in the liver (102.67±35.77 hr), kidney (216.57±130.32 hr), muscle (87.27±14.31 hr), lung (148.50±41.57 hr) and cardiac muscle (269.5±212.11 hr) of the starved turkeys was slightly higher when compared with the half-lives of sulphadimidine in the liver (97.82±56.54 hr), kidney (208.00±139.01 hr), pectoral muscle (77.00±19.27 hr), lung (142.45±16.80 hr) and cardiac muscle (161.70±35.33 hr) of non-starved domestic grower turkeys (Table 7).

Table 7: Half-Lives (Hr) of Intramuscular Sulphadimidine in the Various Tissues of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Tissues	Non-starved turkeys (hr)	Starved turkey (hr)
Liver	97.82±56.54	102.67±35.77
Kidney	208.00±139.01	216.57±130.32
Muscle	77.00±19.27	87.27±14.31
Spleen	96.25±19.30	90.96±47.79
Lung	142.45±16.80	148.50±41.57
Heart	161.70±35.33	269.5±212.11

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. (P>0.05) within and between the groups.

The elimination rate constant of the tissues were not significantly different (p>0.05) between the starved and non-starved domestic grower turkeys (Table 8). However the elimination rate constant was lower in the pectoral muscle (0.008±0.001/hr) of starved turkeys as compared to non-starved turkeys (0.01±0.002/hr).

Table 8: Elimination Rate Constants (1/Hr) of Intramuscular Sulphadimidine in the Various Tissues of Non-Starved and Starved Domestic Grower Turkeys Administered 100mg/Kg Body Weight of Sulphadimidine Intramuscular

Tissues	Non-starved turkeys (1/hr)	Starved turkeys (1/hr)
Liver	0.009±0.003	0.008±0.002
Kidney	0.002±0.001	0.005±0.003
Pectoral Muscle	0.010±0.002	0.008±0.001
Spleen	0.008±0.001	0.011±0.006
Lung	0.005±0.001	0.005±0.001
Cardiac muscle	0.005±0.001	0.009±0.004

Data were presented as mean±standard error of mean (SEM), and analyzed by two way ANOVA using Graph Pad Prism 5.03 for windows followed by Bonferroni post test. (P>0.05) within and between the groups.

4. Discussion

The presence of sulphadimidine residues on the 3rd day post administration of sulphadimidine in the liver, kidney, pectoral muscle, spleen, lung and heart of turkeys shows that sulphadimidine can be absorbed, distributed and resides in tissues of starved and non-starved turkeys in significant quantity when administered intramuscularly. The absorption rate of sulphadimidine is affected by its solubility and birds absorb sulphadimidine rapidly (Shoaf *et al.,* 1987). The presence of sulphadimidine residues between day 3 and 30 at concentration level of 1.27±0.84 ppm and 1.91±1.61 ppm in non-starved turkeys in comparison with the concentration of sulphadimidine residues (1.91±0.83 ppm and 2.55±1.31 ppm) in starved turkeys shows that the starved turkeys retain higher concentrations of the drug. This may be due to dehydration, secondary to starvation. However, the increased level of sulphadimidine between day 3 and 30 in liver, kidney, pectoral muscle, spleen and lung in non-starved and starved turkeys show that sulphadimidine administered via the intramuscular route at 100mg/kg body weight cannot be eliminated from grower turkeys within

30days. However on day 30, the liver of starved turkeys had highest level of sulphadimidine residue (6.5±1.94 ppm) in comparison with that of the kidney (4.3±1.05ppm), pectoral muscle (1.49±0.86 ppm), spleen (0.58±0.58 ppm) and lungs (0.48±0.1 ppm). Also on day 30, the highest concentration of sulphadimidine resided in the liver (5.79±1.86 ppm) of non-starved turkeys in comparison with that of kidney (2.87±1.70 ppm), pectoral muscle (0.99±0.50 ppm), spleen (0.54±0.37 ppm) and lung (0.29±0.00 ppm) respectively, signifying that sulphadimidine reside much more in the liver (the main organ of drug metabolism) than the rest of the organs.

The presence of residues (>0.1 ppm) of sulphadimidine in all the sampled tissues of the non-starved and starved turkeys on day 30 may pose also very high risk of health hazard to consumers. The present findings disagree with the report of Sandhu & Rampal (2006) indicating that sulphadimidine is an intermediate acting sulphonamide that last for 12-24hrs in the body. But on the contrary the present findings agree with the report of Heath et al. (1975) indicating that sulphadimidine residues (0.1-0.4 ppm) could be found in the kidney, liver and spleen of turkeys after 3 days of withdrawal and as long as 14 days, despite the ability of turkeys to metabolize sulphadimidine extensively. In hen, 0.01-0.47 ppm of sulphadimidine residue has been reported (Righter *et al.*, 1971). Variation of withdrawal time of sulphadimidine have been reported as 3 days for broilers (Lüders *et al.*, 1974), 5-6 days for pectoral muscle and 7 days for liver of fattening chickens (Vlahovic *et al.*, 1996) and 4 days for tissues in hen (Nouws *et al.*, 1988).

High tissue levels of sulphadimidine recorded in the present study (>0.2 ppm) poses high risk to consumers. In Australia the minimum residue limit (MRL) of sulphonamide in turkey is 0.2 ppm (NRAAVCA, 2000), while in Europe the MRL of sulphonamide in most edible tissues is 0.1 ppm (Council Regulation, 1990). But the tissue residue of sulphadimidine in liver, kidney, pectoral muscle, heart and brain of rabbit were undetectable after 12days of administration (Etuk *et al.*, 2006). In pig the tissue residue of sulphadimidine was depleted to <0.1 ppm in 4 days and 8 - 10 days in liver and kidney respectively (Duffee *et al.*, 1984). In sheep, sulphadimidine residue was detected at the level <0.14 ppm after three and half days (Bevil *et al.*, 1977). In the present study, the half-life of sulphadimidine residues in the affected organs was 77.00-269.50 hr. This is at variance with the report of Etuk et al. (2006) indicating that the tissue half-life was between 5.63-16.31 hr. This may be due to the difference in the route of administration. However, the most obvious reason for unacceptable residues might be due to failure to keep to the withdrawal period, overdose and long acting drugs (Shearer, 1999). The lack of difference in the half-life and elimination rate constant between residue of sulphadimidine in non-starved and starved turkey showed that starvation does not affect half-life and elimination rate constant of tissue residue of sulphadimidine in turkeys. This agrees with the report of Wang et al. (2006) indicating that starved animals adapt their biochemical and physiological processes to reduce metabolism. The present finding is at variance with the report of Etuk et al. (2006) indicating that rabbit has higher elimination rate constant (0.010-0.23/hr) in comparison with turkeys (0.002-0.01/hr) which may be due to differences in the route of administration, mode of metabolism and elimination.

Higher elimination half-life of sulphadimidine in starved turkeys may also pose risk of environmental contamination, especially when droppings from turkeys administered sulphadimidine is used as organic manure. Watanabe *et al.* (2010) reported that sulphadimidine concentration of ground water was 0.6 ng/l. Sulphadimidine in manure and soil may affect soil microbial and enzyme activities (Caracciolo *et al.*, 2015). For example, it was found to have significant effects on soil respiration with an effective concentration (EC_{10}) of 13 mg/kg in the first 2 days of an experimental test (Liu *et al.*, 2009). Sulphadimidine affects both the functioning (i.e. enzymatic activities) and the structural diversity of a soil microbial community at relatively low antimicrobial concentration, 1-900µg/g (Gutierrez *et al.*, 2010). This signifies that sulphadimidine is a persistent organic pollutant due to its resistance to biological decomposition. It is regularly detected in surface waters even up to 1µg/dm^3 levels, because of their widespread human and veterinary application (Olasehinde *et al.*, 2013, Garcia-Galan *et al.*, 2008).

5. Conclusion

The study concludes that a single dose (100mg/kg body weight) of sulphadimidine via the intramuscular route can cause increased tissue concentration of the drug in the liver, kidney, pectoral muscle, cardiac muscle, lung and spleen of turkeys above the maximum residue limit (0.2 ppm). Therefore, consumption of such tissues should be in excess of 30 days.

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