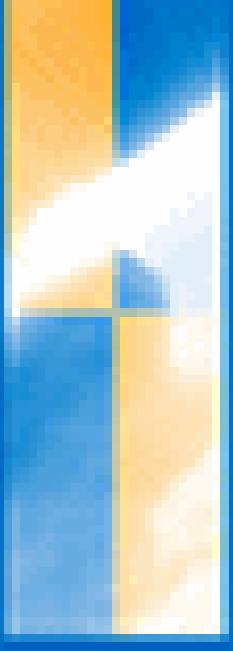
JOURNAL OF
Veterinary Pharmacology and Therapeutics



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# Veterinary Pharmacology and Therapeutics

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# Determination of thiamphenicol residues in albumin and yolk of hen eggs

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(Paper received 3 September 1999; accepted for publication 21 April 2000)

Thiamphenicol is a structural analogue of chloramphenicol with a broad spectrum and similar mechanism of action, which has been shown to be valuable for the treatment of bacterial infections in both animals and humans (Laplassotte and Brunaud, 1961; Yunis *et al.*, 1973; Abdennebi, 1991). Thiamphenicol has a greater *in vivo* activity against pathogenic bacteria than other structural analogues and it is also active against some bacteria that are resistant to chloramphenicol. However, in spite of their chemical similarity, the toxicity of thiamphenicol is lower (reversible dose-related bone marrow suppression) (Yunis *et al.*, 1973; Rankin, 1975).

The presence of thiamphenicol residues in food-producing animals is undesirable from the standpoint of human safety, therefore it was important to develop sensitive methods for its determination. Various methods have been described in the literature for thiamphenicol residue evaluation in several animal species: chickens (Nagata and Saeki, 1991), beef and dairy cattle (Abdennebi *et al.*, 1994), laying hens (Romani *et al.* 1999) and fish (Nagata and Saeki, 1992), but for the determination of thiamphenicol residues in eggs, a sensitive and specific analytical method is required which will separate it from other egg components. The aim of the present study was to develop a rapid, sensitive and specific high performance liquid chromatographic method (HPLC) and to investigate thiamphenicol residues in egg albumin and yolk after administering single and multiple oral doses to laying hens.

Twenty 6-month-old Isabrown laying hens, weighing 1.6–2.00 kg, were individually weighed, randomly housed in seven numbered cages (with water and food available *ad libitum*) such that there were six cages of three animals and one of two animals. Animal care and handling were performed according to the provisions of the EC Council Directive 86/609 EEC, recognized and adopted by the Italian Government (DL 27/1/1992, no. 116).

On the day of the treatment, the birds were allocated into two groups of twelve and eight hens, respectively.

Capsules containing a dose of 40 mg/kg of thiamphenicol base (as the glycinate form, Glitisol®, Zambon, Milan, Italy) were prepared for each hen and administered p.o. to the first group (cages 1–4), while the second group (cages 5–7) received the same oral dose on each of 5 successive days.

Eggs (shell colored) were collected daily for 15 days (total number of eggs 294); yolk and albumin were separated, weighed and stored at −20 °C.

The shells were weighed after washing and 24 h drying at room temperature (20 °C, 60% relative humidity). Shell thickness (with shell membranes) was measured by micrometer (precision 0.01 mm) on acute, obtuse and equatorial regions.

Eggs were collected from five untreated laying hens for blank controls and for preparation of spiked samples.

Sample preparation and drug extraction were carried out according to a method developed previously and partially modified (Romani *et al.*, 1999). Thiamphenicol standards were prepared from pooled blank yolk and albumin by adding known amounts of thiamphenicol to achieve concentrations ranging from 0 to 5 μg/g. Two grammes of albumin or yolk samples were extracted with 6 mL of a phosphate buffer (monobasic potassium phosphate solution 67 mM and anhydrous di-sodium hydrogen orthophosphate solution 67 mM, mixed in a ratio of 4:6 v/v immediately before use) and 20 mL of ethyl acetate. Samples were shaken in a horizontal mixer for 30 min and centrifuged at $5000 \times \boldsymbol{g}$ for 15 min. The organic phase was collected and the hydrophilic phase extracted again with 10 mL of ethyl acetate. The total organic phase was evaporated to dryness in a 40°C rotavapor and the dry residue was dissolved in 1 mL of HPLC mobile phase, which was a mixture of acetate buffer (acetic acid 0.01 M and sodium acetate 0.01 M in double-distilled water) and acetonitrile (8:2 v/v) and 20 μL of this sample were injected into a Jasco HPLC system using a Spherisorb C18 ODS2 column for separation. Ultraviolet absorbance ($\lambda = 230$ nm) was used for drug detection. The retention time for thiamphenicol was 6.30 min and the quantification limit of the method was 10 μg/kg.

Extraction recoveries of thiamphenicol from yolk and albumin were $75.2 \pm 2.0$ and $80.4 \pm 1.1$%, respectively. The precision of the method was expressed as the coefficient of variation

(CV) of the results obtained from different analyses (CV < 8%). The accuracy of the method was definited as the difference between the actual and the theoretical values, expressed according to mean % error (ME < 15%). The linearity of the method was assayed between 10 ng/g and 5 μg/g.

The thiamphenicol residues in both egg components were determined using standard curves by plotting drug concentrations against their corresponding peak area ratio between thiamphenicol standards with extraction and thiamphenicol standards without extraction.

Average yolk and albumin thiamphenicol concentrations vs. time data following a single oral dose of 40 mg/kg of the drug to laying hens are shown in Table 1. Thiamphenicol concentration was not detected in the yolk on the first day and was present in low concentration on the second day (10.50 ± 3.57 μg/kg). On the sixth day, a peak drug concentration was obtained (131.86 ± 25.89 μg/kg); then it decreased over time to become undetectable on day 11.

Thiamphenicol residues in albumin reached the maximum value of 309.36 ± 49.86 μg/kg on the first day, then declined rapidly to 13.71 ± 7.79 μg/kg 48 h after administering a single dose.

Data on the concentration of thiamphenicol in yolk and albumin after repeated oral doses are given in Table 2. Drug levels in yolk began to rise on the second day of treatment reaching a peak of 356.64 ± 86.23 μg/kg on the third day after the end of treatment (day 8) and decreasing gradually during the following days to become undetectable on day 14.

After multiple doses, albumin levels of thiamphenicol reached a peak concentration on the third day of treatment. High values of thiamphenicol were observed during the five days of treatment, then thiamphenicol concentration declined significantly on the sixth day and it was not detected on day 7.

No differences in yolk, albumin, shell weights, and shell thickness were observed between eggs layed by control and treated hens (12.9 ± 1.11 g, 35.6 ± 3.80 g, 5.5 ± 0.53 g and 37.5 ± 2.58 mm/100 vs. 12.5 ± 0.9 g, 35.8 ± 3.5 g, 5.6 ± 0.6 g and 36.9 ± 3.0 mm/100, respectively; $P > 0.05$).

**Table 1.** Thiamphenicol concentrations (μg/kg; mean ± SD) in egg yolk and albumin following a single oral dose (40 mg/kg) in laying hens

| Day | *n* | Yolk | Albumin |
|---|---|---|---|
| 1 | 11 | n.d. | 309.36 ± 49.86 |
| 2 | 14 | 10.50 ± 3.75 | 13.71 ± 7.79 |
| 3 | 11 | 31.07 ± 8.75 | n.d. |
| 4 | 12 | 50.50 ± 12.21 | |
| 5 | 12 | 92.36 ± 48.61 | |
| 6 | 12 | 131.86 ± 25.89 | |
| 7 | 10 | 79.64 ± 21.87 | |
| 8 | 11 | 42.35 ± 10.33 | |
| 9 | 12 | 28.21 ± 8.61 | |
| 10 | 10 | 11.79 ± 5.23 | |
| 11 | 12 | n.d. | |

*n* = number of eggs.
n.d. = not detected.

**Table 2.** Thiamphenicol concentrations (μg/kg; mean ± SD) in egg yolk and albumin following multiple oral doses (40 mg/kg/day for 5 days) in laying hens

| Day | *n* | Yolk | Albumin |
|---|---|---|---|
| 1 | 8 | n.d. | 324.29 ± 47.85 |
| 2 | 8 | 44.07 ± 15.49 | 326.76 ± 50.00 |
| 3 | 8 | 83.00 ± 26.02 | 372.50 ± 60.98 |
| 4 | 7 | 157.64 ± 45.31 | 310.93 ± 41.11 |
| 5 | 8 | 226.93 ± 64.56 | 344.07 ± 59.21 |
| 6 | 7 | 289.99 ± 69.31 | 29.71 ± 10.36 |
| 7 | 8 | 347.14 ± 78.40 | n.d. |
| 8 | 8 | 356.64 ± 86.23 | |
| 9 | 7 | 277.89 ± 80.31 | |
| 10 | 9 | 251.79 ± 61.82 | |
| 11 | 8 | 181.86 ± 35.11 | |
| 12 | 9 | 79.43 ± 23.2 | |
| 13 | 7 | 31.57 ± 10.45 | |
| 14 | 7 | n.d. | |

*n* = number of eggs.
n.d. = not detected.

In this study the proposed HPLC method was rapid, sensitive and specific for the determination of thiamphenicol in the albumin and yolk in hen eggs.

Thiamphenicol yolk levels obtained after single oral administration rose slowly to a maximum value and the drug was not detected on day 11, while albumin accumulated the drug rapidly, the drug also depleted rapidly from albumin and on the third day after treatment no residue was detected. A similar drug depletion pattern in albumin and yolk was found in a previous study of chloramphenicol detection performed in eggs from laying hens to which chloramphenicol was given at a concentration of 800 mg/kg of food for 24 h (Samouris *et al.*, 1993). The concentrations obtained in yolk after multiple doses of thiamphenicol reached values nearly three times higher than those obtained following single dose and a maximum concentration was present on day 3 after terminating the treatment. In albumin, peak drug levels were constant during the five-day treatment period, decreasing rapidly on day 6 and were not detected on day 7.

These results indicate that thiamphenicol residues persist for a longer period in yolk than in albumin, justified by the fact that albumin is produced and excreted in 24 h, while the turnover time for yolk is 6–8 days (Sauveur, 1988).

The low thiamphenicol concentrations observed in a previous study in laying hens' oviducts 24 h after a single dose, suggest that the drug can rapidly enter in the albumin (Romani *et al.*, 1999).

In conclusion, the present results demonstrate the pattern of thiamphenicol residues in yolk and albumin of eggs following oral administration of a single dose and multiple doses over a 5-day period. Residues (> 10 μg/kg) persisted longer in yolk than in albumin and were present in yolk for 10 days after administering a single dose and for 8 days after terminating the administering

of multiple (five) doses. After multiple doses, the values of thiamphenicol concentrations were under the limit of detection of the method at the ninth day after the end of the treatment.

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