Journal of Animal and Veterinary Advances 11 (7): 1012-1017, 2012
ISSN: 1680-5593

Residue Depletion of Florfenicol and its Metabolite Florfenicol Amine in Eggs

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Abstract: Eggs of laying hens were used to study residue depletion of Florfenicol (FF) and its metabolite Florfenicol Amine (FFA) after oral dose (25.0, 50.0 and 100.0 mg kg^{-1} body weight), once daily for 5 days. The residues were determined by High Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD). The maximum concentration of FF was all detected at 1 day in the albumen, yolk and whole eggs; the maximum concentration of FFA was detected at 1 day in the albumen and whole eggs, at 3 days in the yolk after cessation of medication. After oral administration of 100.0 mg kg^{-1}, concentrations of FF and FFA were all below the LODs (1.5 μg kg^{-1} for FF and 0.5 μg kg^{-1} for FFA) at 7 days for the albumen, 11 days for yolk and 10 days for whole egg after cessation of medication.

Key words: Florfenicol, florfenicol amine, residue depletion, eggs, yolk, medication

INTRODUCTION

FF, a fluorinated derivative of Thiamphenicol (TAP), is a synthetic broad-spectrum antibiotic used in veterinary medicine belonging to the family of agents that include Chloramphenicol (CAP) and TAP (Papich and Riviere, 2001) which has a fluorine atom instead of the hydroxyl group located at C-3. Therefore, it does not carry the risk of inducing human aplastic-anemia (Kowalski *et al*., 2005).

Furthermore, FF prevents bacterial enzymatic acetylation; consequently this product has more antibacterial activity than CAP and TAP (Cannon *et al*., 1900). So, FF is believed to be an ideal replacement of these two drugs. Because of its broad-spectrum antibacterial and absorbed rapidly, it has been widely used in veterinary clinic to treat bacterial diseases. (Booker *et al*., 1997; Jim *et al*., 1999; Angelos *et al*., 2000). FF is characterized by high bioavailability, good tissue penetration and rapid elimination which are important for systemic treatment of animals in the food production industry.

In eggs of laying hens, the residues of FF not only include parent drug and metabolites include FFA, florfenicol alcohol, florfenicol oxamic acid and monochloroflorfenicol. FFA is the longest-lived major metabolite in the eggs from laying hens. The European Union (EU) has been set the tolerance level for these compounds as the Maximum Residue Limits (MRLs) to ensure safety for the food production industry. The value of MRLs was calculated by the sum of FF and its metabolites. The sum of florfenicol and its major metabolite florfenicol amine (200 μg kg^{-1}) in swine muscle by China and EU and have also been set for the sum (100 μg kg^{-1}) of florfenicol and its major metabolite florfenicol amine in foodstuffs of animal origin by China, USA and EU. The total residue of FF and FFA were not exceeding 100 μg kg^{-1} in poultry. So far, China, USA and EU have not given MRLs value for florfenicol in eggs.

At present, although the method of determination of FF and its metabolite FFA had been reported on domestic and abroad (Nagata and Saeki, 1992; Hormazabal *et al*., 1993; Pfenning *et al*., 2000; Wrzesinski *et al*., 2003; Ding *et al*., 2005) and the studies about residue depletion

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of FF and FFA had been reported (Boon *et al*., 1991; Horsberg *et al*., 1994; Wrzesinski *et al*., 2006; Feng *et al*., 2008; Sun *et al*., 2010) but in eggs of laying hens has scarcely been documented.

In domestic breeding conditions this study was undertaken to investigate residue depletion of FF and its metabolite FFA in albumen, yolk and whole eggs of laying hens following oral dose (25.0, 50.0 and 100.0 mg kg^{-1} body weight), once daily for 5 days and provides data for a more prudent use of FF in eggs.

MATERIALS AND METHODS

Animals: The study was undertaken in accordance with the ethics requirements and authorized by the official ethical committee of the university. One hundred laying hens were obtained from a poultry breeding farm (Jinghai, Jiangsu province, China) aged 28 weeks and weighting 1.75±0.20 kg which were used in this study. The hens were housed in individual stainless steel cages. Hens were weighed and marked before administration. The hens were feed about 7 days before to study. During this period, they were fed drug-free assorted feed *ad libitum* with free access to water.

Experiments: The laying hens were randomly divided into 4 groups (A-D) of 25 hens per group. The group A did not receive any treatment and were used to determine the validation criteria of the analytical method. The group B-D were used to investigate residue depletion of FF and its metabolite FFA after oral administration of FF capsules 25.0, 50.0 and 100.0 mg kg^{-1} body weight, given once daily for 5 consecutive days, respectively. All dosages were administered at 8:00-9:00. The eggs were collected daily at 17:30-18:00 from the beginning working day to cessation of treatment for 20 days.

Analytical method: The albumen, the yolk and whole eggs were separated and homogenized using a tissue homogenizer (ART MICCRA D-9, ART-moderne Labortechnik e.k., Germany). A 3.0 g of homogenized samples was accurately weighted into a 50 mL polypropylene centrifuge tube. The sample was vortexed for 30 sec, 1 mL acetonitrile-water (30:70, v/v) was added to the tube.

After the 8 mL of ethyl acetate-acetonitrile-ammonium hydroxide (49:49:2, v/v) was added using a vortex mixer (Model G560E, Scientific Industries Inc.,USA) and the mixture was vortexed for 2 min and homogenized ultrasonically for 15 min then centrifuged for 10 min at 8000×g. The supernatant was removed and transferred to a 20 mL glass test tube and the extraction step was repeated. The supernatants were combined and evaporated to dryness under a gentle nitrogen stream at 50°C (N-EVAP 111, Organization Associates Inc., Berlin, MA, USA). The dried residue was reconstituted in 0.5 mL acetonitrile and vortexed. Then, 8 mL of hexane was added into the tube and well mixed and allowed to stand for 5 min. After centrifugation for 5 min at 3500×g, the hexane layer was discharged. This de-fatting step was repeated. The extraction was evaporated to dryness under a gentle stream of nitrogen at 50°C and then was stable at -4°C. Before analysis, the residue was dissolved in 1 mL of the mobile phase solution. Then the solution was transferred to a 1.5 mL centrifuge tube, vortexed. After centrifugation for 15 min at 12100×g, the resulting solution was filtered through a 0.22 μm filter. Then, 200 mL of the supernatants was injected manually into the HPLC system. Chromatography was performed on a Waters Alliance 515 LC System and a Waters multi λ 2475 fluorescence detector (Waters Corp., Milford, MA, USA). The fluorescence detector of HPLC was set at 224 nm for excitation wavelength and 290 nm for emission wavelength.

The separation was achieved on a LiChrospher C_{18} column (250×4.6 i.d. and 5 μm; Merck KGaA). The column temperature was maintained at 30°C. The injection volume was 200 μL manually with a 200 μL quantitative ring. The analysis was carried out using acetonitrile (A), 0.01 M sodium dihydrogen phosphate containing 0.005 M sodium dodecyl sulfate and 0.1% triethylamine, adjusted to pH 4.8 with 85% phosphoric acid (B) (A/B, 35:65 v/v) as the mobile phase, at a flow rate of 1.0 mL min^{-1}. The mobile phase was prepared daily and prepared daily before use. The sensitivity of the method was assessed by LODs (1.5 for FF and 0.5 μg kg^{-1} for FFA) and LOQs (5.0 for FF and 2.0 μg kg^{-1} for FFA). The LODs was defined by the concentration of each of the two analytes in the sample matrix giving a signal-to-noise ratio of 3:1. The LOQs was defined as the lowest point on the calibration curve for each of the two analytes based on a signal-to-noise ratio of 10:1. The chromatograms from blank eggs and spiked with FF and FFA are shown Fig. 1.

The method of analysis was validated by recovery experiments performed on the albumen, yolk and whole eggs spiked with known concentrations of FF and FFA. The spiked samples was analysed by the method used in the study. The mean extraction recoveries of FF ranged from 84.7-96.9% for the albumen, 74.36-85.13% for the yolk and 87.41-92.29% for whole eggs. The mean extraction recoveries of FFA were from 83.71-97.14% for the albumen, 79.61-97.23% for the yolk and 89.01-95.15% for whole eggs.

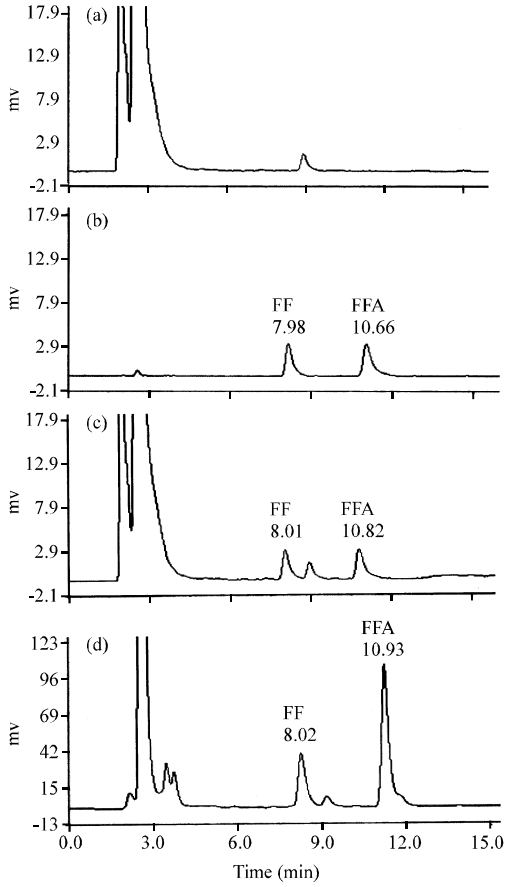

Fig. 1: HPLC chromatograms of FF and FFA; a) extract of blank egg; b) FF (0.357 μg mL^{-1}) and FFA (0.125 μg mL^{-1}) standard; c) egg spiked with FF and FFA and d) egg after oral administration of FF

RESULTS AND DISCUSSION

Under the optimized chromatographic conditions, the retention time of FF and FFA was 7.98 and 10.66 min, respectively (Fig. 1). No interference was observed at the retention time of the analytes.

The residues of FF and its metabolite FFA in the albumen, the yolk and whole eggs after oral administration of FF capsules (25.0, 50.0 and 100.0 mg kg^{-1} body weight, once daily for 5 consecutive days) were determined; concentrations of FF residues are shown in Table 1. The maximum concentration of FF was all detected at 1 day after cessation of medication in the albumen, the yolk and whole eggs. At the early days after cessation of medication, concentrations of FF in the albumen, the yolk and whole eggs were eliminated faster but they were eliminated slowly at the later period, concentrations of FF in the albumen were higher than those in the yolk at administration period, it was in contrast at the withdrawal period. Concentrations of FF in the whole eggs were

Table 1: Residues (μg kg^{-1}) of FF in albumen, yolk and whole eggs (mean±SD, n = 6) during and after oral treatments at 25.0 mg kg^{-1} body weight (group B) and 50.0 mg kg^{-1} body weight (group C) and 100.0 mg kg^{-1} body weight (group D)

			Residues (μg kg^{-1})		
Groups	Period	Time (days)	Albumen	Yolk	Whole egg
	Administration	1	ND	ND	ND
		2	225.5±6.3	117.8±14.2	177.0±49.6
		3	285.7±64.3	208.5±7.9	250.9±45.3
		4	309.0±84.7	228.5±54.4	272.7±53.8
		5	320.2±36.4	283.6±31.8	303.7±53.3
B	Withdrawal	1	335.4±60.0	378.4±47.7	354.7±34.7
		2	65.4±33.6	234.6±35.0	141.5±32.5
		3	22.4±5.4	198.9±69.9	101.8±16.1
		4	6.7±2.0	59.9±2.4	27.5±1.9
		5	ND	22.3±6.4	ND
		6	ND	8.9±4.7	ND
		7	ND	ND	ND
		8	ND	ND	ND
		9	-	ND	-
	Administration	1	ND	ND	ND
		2	305.7±175.3	228.3±6.3	271.4±18.4
		3	454.5±223.5	352.9±64.3	407.8±91.2
		4	591.7±125.0	483.0±84.7	541.8±176.5
		5	640.5±91.9	643.9±36.4	641.1±113.3
C	Withdrawal	1	662.0±149.8	757.3±60.1	702.3±67.5
		2	204.0±27.6	646.2±33.6	410.5±68.7
		3	71.3±11.3	578.9±5.4	284.8±22.9
		4	34.1±9.6	530.3±2.0	247.6±87.2
		5	18.0±6.4	327.2±14.2	153.2±65.6
		6	ND	158.3±7.5	74.5±18.8
		7	ND	85.4±4.6	37.4±6.9
		8	ND	46.2±2.8	20.4±7.5
		9	-	17.5±7.2	ND
		10	-	ND	ND
		11	-	ND	ND
		12	-	ND	-
	Administration	1	ND	ND	ND
		2	1131.4±43.6	831.9±26.3	996.6±91.8
		3	1500.7±114.1	952.8±64.3	1254.1±108.7
		4	1625.6±45.8	1437.8±84.7	1541.1±121.9
		5	1739.2±93.6	1612.5±36.4	1682.2±87.6
D	Withdrawal	1	1803.1±107.2	1810.4±60.0	1806.4±142.8
		2	439.5±87.4	1684.7±33.6	999.8±93.6
		3	192.1±69.8	1443.1±25.4	755.1±91.2
		4	84.3±5.4	912.0±22.0	456.8±46.2
		5	40.6±4.2	488.9±24.2	242.3±66.1
		6	19.1±5.1	163.4±74.3	84.0±11.2
		7	ND	134.7±23.5	60.6±29.4
		8	ND	63.9±14.2	28.7±5.2
		9	-	32.3±8.5	14.5±4.3
		10	-	10.3±7.7	ND
		11	-	ND	ND
		12	-	ND	-

ND: Not Detected, -: Not Detection

throughout between the albumen and the yolk. The concentrations of FF depleted much slower from the yolk than the albumen and whole egg.

The concentrations of FFA in eggs of laying hens were shown in Table 2, concentrations of FFA in the albumen, the yolk and whole egg were eliminated faster but they were eliminated slowly at the later period. At administration period, concentrations of FFA in the yolk were higher than those in the albumen. At withdrawal period, the concentrations of FFA in the yolk were higher than those in the albumen too. The concentrations of FFA

Table 2: Residues (μg kg^{-1}) of FFA in albumen, yolk and whole eggs (mean±SD, n = 6) during and after oral treatments at 25.0 mg kg^{-1} body weight (group B) and 50.0 mg kg^{-1} body weight (group C) and 100.0 mg kg^{-1} body weight (group D)

			Residues (μg kg^{-1})		
Groups	Period	Time (days)	Albumen	Yolk	Whole egg
B	Administration	1	ND	ND	ND
		2	16.6±4.0	26.5±6.4	21.1±8.9
		3	37.7±4.9	115.3±15.4	72.6±15.7
		4	71.9±13.7	229.7±142.9	142.9±66.8
		5	126.6±24.3	269.0±76.7	190.7±85.8
	Withdrawal	1	275.9±51.2	307.7±45.6	290.2±78.1
		2	93.2±7.8	353.3±67.8	205.2±68.7
		3	6.2±2.2	388.7±58.7	126.7±32.7
		4	ND	94.1±14.8	20.8±19.4
		5	ND	29.6±19.0	ND
		6	ND	21.8±10.3	ND
		7	-	5.3±2.5	ND
		8	-	ND	-
		9	-	ND	-
		10	-	ND	-
C	Administration	1	ND	ND	ND
		2	29.9±4.0	41.4±4.0	34.1±2.3
		3	42.9±8.3	286.7±4.9	142.6±49.5
		4	105.6±19.2	464.8±52.7	287.2±92.4
		5	153.8±53.4	635.7±224.3	358.7±86.5
	Withdrawal	1	368.6±137.3	740.2±51.2	538.5±102.9
		2	84.2±43.0	865.8±7.8	508.7±88.5
		3	36.2±8.7	950.5±36.4	432.6±91.5
		4	8.8±1.3	855.5±43.7	376.8±31.8
		5	ND	617.3±75.2	237.6±64.3
		6	ND	265.3±34.5	118.0±34.0
		7	ND	100.8±58.4	46.3±1.9
		8	-	48.0±12.7	21.7±3.7
		9	-	29.2±8.5	13.1±2.5
		10	-	ND	ND
		11	-	ND	ND
		12	-	ND	ND
D	Administration	1	ND	ND	ND
		2	62.2±40.7	478.9±34.3	249.7±64.2
		3	89.5±48.3	550.6±64.9	297.0±84.4
		4	161.9±35.3	826.9±52.7	461.2±86.1
		5	357.6±45.7	1326.8±74.3	793.7±86.5
	Withdrawal	1	596.4±65.8	1549.0±51.2	1025.1±90.7
		2	377.6±24.4	1774.0±87.8	1006.0±101.2
		3	79.7±18.6	2028.2±94.2	950.5±75.1
		4	54.7±7.2	1573.3±98.4	709.6±90.5
		5	20.1±2.0	809.0±83.2	375.1±73.4
		6	7.6±4.6	356.9±53.7	164.8±19.1
		7	ND	207.8±54.5	116.0±20.7
		8	ND	97.3±22.3	43.8±13.6
		9	ND	40.4±18.4	17.2±7.1
		10	-	6.2±2.8	ND
		11	-	ND	ND
		12	-	ND	ND
		13	-	ND	-

ND: Not Detected, -: Not Detection

depleted much slower from the yolk than the albumen and whole eggs. The maximum concentration of FFA was also detected at 1 day in the albumen and whole eggs, at 3 days in the yolk after cessation of medication.

After oral administration of 25.0 mg kg^{-1}, concentrations of FF were below the LODs at 5 days for the albumen, 7 days for the yolk and 5 days for whole egg after termination of FF treatment and concentration of FFA were below the LODs at 4 days for the albumen, 8 days for the yolk and 5 days for whole eggs after termination of FF treatment. After oral administration of 50.0 mg kg^{-1}, concentrations of FF were below the LODs at 6 days for the albumen, 10 days for the yolk and 9 days for whole eggs after termination of FF treatment and concentrations of FFA were below the LODs at 5th days for the albumen, 10 days for the yolk and whole eggs after termination of FF treatment. For oral administration of 100.0 mg kg^{-1}, concentrations of FF and FFA were all below the LODs at 7 days for the albumen, 11 days for the yolk and 10 days for whole egg after cessation of medication in eggs. Concentrations of FF and FFA in the albumen, the yolk and whole eggs were positively correlated with FF orally administered dose.

In this study, concentrations of FF were detected in the albumen, the yolk and whole eggs at administration period and withdrawal period, after the laying hens orally administered successively FF capsules of 25.0, 50.0 and 100.0 mg kg^{-1} body weight, once daily for 5 consecutive days. A study on residue depletion of FF and its metabolite FFA was conducted in the albumen, the yolk and whole eggs of laying hens. The results of experiment indicated that concentrations of FF in the albumen, the yolk and whole eggs have a rise trend and concentrations of FF at the early days were eliminated faster than the later period after cessation of medication. The maximum concentration of FF was detected at 1 day after cessation of medication in the albumen, the yolk and whole eggs this maybe due to the FF rapidly incorporated into the eggs. At administration period, the concentrations of FF in the albumen were higher than those in the yolk this maybe due to the yolk formation first than the albumen and the relatively high concentration of FF in the yolk was a reflection of serum concentration and depended on the concentration of FF in eggs on the before laying. At withdrawal period, concentrations of FF in the yolk were higher than those in the albumen, it indicate that concentrations of FF in the albumen were eliminate faster than those in the yolk and concentrations of FF in the yolk was eliminated which need a long time and may be explained by the lipid solubility of the drug (Posyniak *et al*., 1996). The concentrations of FF in the albumen, the yolk and whole eggs were positively correlated with FF orally administered doses.

Researchers found that concentrations of FFA were detected in the albumen, the yolk and whole eggs at administration period and withdrawal period after oral administration. The results of experiment indicated that residues of FFA in the albumen, the yolk and whole eggs also have a rise trend. At the early days after cessation of medication, concentrations of FFA in the albumen, the

yolk and whole eggs were eliminated faster but they were eliminated slowly at the later period. Concentrations of FFA in the albumen, the yolk and whole eggs were positively correlated with FF orally administered doses. The maximum concentration of FFA was detected at 1 day in the albumen and whole eggs, at 3 days in the yolk after cessation of medication. At administration period, concentrations of FFA in the yolk were higher than those in the albumen because of FF poorly soluble in aqueous solutions and its lipophilicity, FF shows a good tissue penetration (Anadon *et al*., 2008). At withdrawal period, the concentrations of FFA in the yolk were higher than those in the albumen too. Concentrations of FFA in the albumen were eliminating faster than those in the yolk and residues of FFA in the yolk were eliminated need a long time.

The prolonged of residues of FF and FFA in the eggs can play an important role in human food safety because the compounds could give rise to a possible health risk. A withdrawal time of 6 days was necessary to ensure that the residues of FF were less than the Maximal Residue Limits (MRLs) or tolerance established by the EU. The present work is the first to describe the residue depletion of FF and its major metabolite FFA in the albumen, the yolk and whole egg using a validated HPLC with fluorescence detection.

CONCLUSION

The study provides data for a more prudent use of FF in eggs, suggesting a possible rational dosing after treatment to guarantee safety in foods for the consumers. Researchers suggest the withdrawal time are 4 days for albumen, 8 days for the yolk and 5 days for whole eggs after the laying hens orally administered successively FF capsules of 25.0 mg kg^{-1} body weight, once daily for 5 consecutive days.

Researchers suggest the withdrawal period are 5 days for the albumen, 10 days for the yolk and whole eggs after the laying hens orally administered successively FF capsules of 50.0 mg kg^{-1} body weight, once daily for 5 consecutive days. Researchers suggest the withdrawal period are 7 days for the albumen, 11 days for the yolk and 10 days for whole eggs after the laying hens orally administered successively FF capsules of 100.0 mg kg^{-1} body weight, once daily for 5 consecutive days.

ACKNOWLEDGEMENT

This research was supported by the High-Tech Project in Jiangsu province (BG2005307) and by the Special Fund Project of Jiangsu province for the Transformation of Scientific and Technological Achievements (BA2008071).

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