Research Notes

Oxytetracycline Transfer into Chicken Egg Yolk or Albumen

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ABSTRACT This study was conducted to determine whether the approved doses of oxytetracycline (OTC) for breeder hens and meat-type poultry would produce drug residue transfer into egg components when fed to laying hens. Twenty hens were assigned to equal groups (n = 10) and fed either 50 or 200 g/ton OTC for 5 d. Oxytetracycline concentrations in egg components were determined daily during a 2-d pretreatment control period, the 5-d dosing period, and following drug withdrawal. The stability and drug content of the medicated feed were determined the day dosing was started and the day of withdrawal. Residues of OTC were not detectable during the predosing, dosing, or withdrawal period in egg yolks. Oxytetracycline residues were detectable, however, in egg albumen during the 5th d of treatment and the 1st d of medicated feed withdrawal. These concentrations were close to the limit of the assay's sensitivity (117 ppb). These data indicate that illegal or unintentional dosing of laying hens with feed medicated at the doses allowed for breeder hens or meat-type poultry should not produce consistently detectable levels of residues of OTC in eggs.

(*Key words*: drug residues, oxytetracycline, eggs, yolk, albumen)

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INTRODUCTION

Oxytetracycline (OTC) is currently approved for use in feed to increase egg production and feed efficiency and for the prevention of disease for breeder hens and meat-type poultry (Code of Federal Regulations, 1998). Oxytetracycline is not approved for use in laying hens producing eggs for human consumption. However, eggs may contain OTC residues after: 1) illegal or extra-label use of drugs, 2) use of feed unintentionally cross-contaminated during feed mixing, 3) use of mislabeled broiler feed in laying hens.

Previous research has shown that tetracyclines are deposited in whole eggs and egg components when fed to laying hens (Katz *et al*., 1973; Yoshida *et al*., 1973; Roudaut *et al*., 1987a; Yoshimura, *et al*., 1991). These studies did not evaluate the potential for OTC transfer into egg components following the misuse or unintentional dosing of laying hens with medicated feed at the approved doses (50 or 200 g/ton) for meat-type poultry. The purpose of this study was to evaluate OTC transfer into egg albumen and yolk when hens are fed medicated feed dosed for breeder hens or meat-type poultry.

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MATERIAL AND METHODS

Animals

A total of 20 Single Comb White Leghorn hens, 56 wk of age, were used in this study. Hens were individually caged and had *ad libitum* access to either standard laying hen feed or medicated feed and water and subjected to 14 h of light daily.

Experimental Procedure

Twenty hens were assigned to groups (n = 10) and subjected to a 2-d predosing period, a dosing period receiving either 50 or 200 g/ton for 5 d and a withdrawal period until OTC residues were no longer detectable in egg components. Eggs were collected and albumen and yolks were separated, and approximately 10 g of either the albumen or yolk homogenate were diluted 1:3 with 0.1 *M* monopotassium phosphate buffer (pH = 4.5). These solutions were vortexed and centrifuged at 1,500 × *g* for 30 min at 5 C. The supernatant was transferred and stored at –20 C until analysis.

Medicated feed was prepared by the addition of a 10% OTC, 90% soybean meal premix to the standard layer ration. After mixing for 10 min in a paddle mixer, samples were taken from the top, middle, and lower layers of feed

Abbreviation Key: OTC = oxytetracycline.

for determination of OTC content and uniformity in the feed.

Assay Plate Preparation

Samples were assayed using an agar diffusion microbiological method (Roudaut *et al*., 1987a) with modifications (Donoghue *et al*., 1996). Petri dishes (100 mm in diameter) were filled with 8 mL of agar and six cylinders (8 × 10 mm) were evenly placed on the agar. The agar (antibiotic medium 8)[2] was inoculated with spores of *Bacillus cereus* ATCC 11778[2] at a concentration of 5×10^3 spores per milliliter of agar.

Assay Procedure

Standard Curve. A matrix matched standard curve was constructed by spiking control feed, yolk, or albumen with OTC. These spiked standards were treated identically to unknown samples. Each standard concentration was pipetted into three plates, three alternate cylinders were filled with the standard (200 μL) and the other three cylinders were filled (200 μL) with a reference concentration. The overall reference concentration falls within the range of the standard curve. Triplicate plate averages for each standard point were corrected to the overall reference concentration. Reference values may also have been used as a standard point. A best fit regression line using the diameter of growth inhibition zones (millimeters: Fisher Zone Reader) was calculated by the method of least squares. The assays were incubated at 30 ± 1 C for 16 to 18 h. The lower limit of assay sensitivity was calculated to be 26, 258, or 117 ng/g (ppb) for feed, yolk or albumen samples, respectively.

Sample Procedure. Each sample was pipetted into a plate. Three alternate cylinders were filled with the sample (200 μL) and the other three cylinders were filled (200 μL) with a reference concentration. Each plate was corrected to the overall reference concentration.

RESULTS

There was no detectable OTC found in egg yolk of hens dosed with 50 and 200 g OTC/ton. In albumen, the majority of samples also had no detectable OTC residues (Table 1). Only two hens on Day 5 of treatment (Hens 18 and 20; 200 g/ton dose) and one hen on the 1st d of drug withdrawal (Hen 18; 200 g/ton dose) had detectable levels of OTC in albumen of eggs laid those days. These levels were close to the limit of assay sensitivity of 117 ppb.

Medicated feed samples assayed out at 81, 84, and 81% or 94, 89, and 91% for OTC from the top, middle, and lower layers of medicated feed after mixing for the 50 or 200 g/ton doses, respectively. These assay values were determined on the 1st d of dosing hens and averaged 82 and 91% of predicted OTC content in the 50 or 200 g/ton medicated feed, respectively. On the day of medicated feed withdrawal, the average OTC content of the medicated feed was 79 and 91% of predicted OTC levels for the 50 and 200 g/ton medicated feed.

TABLE 1. Oxytetracycline (OTC) transfer from the feed into egg yolk or albumen following a 5-d dosing period with either 50 or 200 g/ton OTC-medicated feed

		OTC Content	
Period	Days	Yolk	Albumen
		(ppb)	
Predose	1	ND[1]	ND
	2	ND	ND
Dosing	1	ND	ND
	2	ND	ND
	3	ND	ND
	4	ND	ND
	5	ND	120[2]
Withdrawal	1	ND	100[3]
	2	ND	ND
	3	ND	ND
	4	ND	ND
	5	ND	ND
	6	ND	ND

[1]ND equals not detectable (assay sensitivity of 258 or 117 ppb for yolk or albumen, respectively).

[2]Only 2 out of 10 hens dosed with 200 ppm OTC in the feed produced detectable levels.

[3]Only 1 out of 10 hens dosed with 200 ppb OTC in the feed produced detectable levels. Although this hen's sample produced readable zones on the assay plate, after accounting for reference zone sizes, the sample concentration was mathematically calculated to be lower than the limit of assay detection.

DISCUSSION

Oxytetracycline is not approved for use in layer hens and no official violative residue level has been established for egg components (Code of Federal Regulations, 1998). Violative levels for the sum of all tetracycline residues (oxytetracycline, chlortetracycline and tetracycline) have been established for other edible poultry tissues at 2 ppm for muscle, 6 ppm for liver, and 12 ppm for fat and kidney (Code of Federal Regulations, 1998). Results from this study indicate that the misuse, illegal, or unintentional feeding of OTC-medicated feed using the approved meat-type doses, when fed for 5 d to laying hens, produces OTC transfer into edible egg components at the lower end or below the sensitivity of our method (117 or 258 ppb for albumen or yolk, respectively). These results are supported, in part, by the studies of (Katz *et al*., 1973; Yoshida *et al*., 1973; Rouduat, *et al*., 1987a; Yoshimura *et al*., 1991). Although these authors used different doses or routes of administration (water, feed, or injected), the ratio of the amount of OTC given vs observed in whole egg is within the realm of what we observed in this study.

Unfortunately, examination of just the whole egg may mask potentially high levels of drug residues or other

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contaminants in either egg yolk or albumen. A number of studies have demonstrated that many drugs are preferentially deposited in either yolk or albumen (Arnold and Somogyi, 1985; Roudaut *et al*., 1987b; Corpet *et al*., 1988; Roudaut and Moretain, 1990; Yoshimura, *et al*., 1991; Nagata *et al*., 1992; Petz, 1993; Donoghue *et al*., 1994; Kan *et al*., 1996; Keukens *et al*., 1996). Examination of whole eggs exclusively may mislead investigators evaluating residue content resulting in a dilution effect of one edible matrix on the other. This dilution effect is especially true in the case of residues deposited in egg yolk. Because egg albumen makes up approximately two-thirds the edible egg mass, it is possible that drug concentrations in egg yolk will be diluted threefold by uncontaminated albumen. As many drugs preferentially transfer into egg yolk, this dilution is a real possibility. Due to the separation of egg yolk and albumen for use in further processed foods, it is important to evaluate each of these components individually to accurately assess residue concentrations and concomitant toxicity potentials. In the present study, even after examining individual egg components, OTC concentrations did not exceed the tolerance established for other edible poultry tissues.

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