EXCRETION OF OXYTERACYLINE IN EGGS AFTER ORAL ADMINISTATION

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Abstract. *The residue oxytetracycline (OTC) in eggs of laying hens can be determined through a microbiological method of diffusion in agar after oral therapy with an esophageal probe with a dose (100 mg / kg for 5 days). The sensitivity of the method is evaluated to 0.08 µg / g in albumen and 0.27 µg / g yolk. The duration of the elimination of OTC in the albumen and yolk is respectively equivalent to 6 and 7 days after stopping treatment.*

Keywords: excretion, residues, oxytetracycline, eggs.

INTRODUCTION

Eggs are a commodity of great importance for human consumption. In Algeria, every year, about an average of 120 eggs are per consumed individual [4]. The vast bulk of these eggs is consumed in processed foods such as ice cream, baked goods, etc. ... [2]. The majority of these eggs came from hens farms, which undergo various treatments with antibiotics administered by some routes, for therapeutic, preventive and primarily to improve growth, yield and production of eggs [10]. Although these uses are beneficial, antibiotic therapy has two major drawbacks:

The creation of antibiotic-resistant bacterial strains in laying hens. The possibility of contamination of the egg residues, whose impact on the human health may be of concern if the limited date or the waiting times are not met (time limit between the last dose of the antibiotic and when it no longer has any residue in its production of eggs) [7]. The impact of residues on animal and human health is of great importance to lawmakers and concerned global organizations, urging them to determine the maximum concentration that can be consumed without health hazard [8]. Unfortunately, no legislation in Algeria was introduced in relation to this problem. This absence of concern on antibiotic residues in eggs has been developed; this prompted us to study this problem in order to establish tolerances and waiting to meet for veterinary antibiotics used in the field.

MATERIAL AND METHOD

Breeding conditions: The experiment was conducted at Regional Veterinary Laboratory in Tizi Ouzou on 20 laying hens bred ISABROWN (the most common in Algeria), with an average weight of 1.9 kg, divided into 2 groups (experimental and witness), a pet at room temperature (22 ° C), day length was 14 hours / day. The birds aged 52 weeks received a diet based on "laying flour high-energy free of antibiotics, and water at will. Experimentation: OTC was administered daily at the same time, using an oesophageal probe with a dose of 100 mg / kg / day for 5 days.

The eggs of each batch of chickens were collected daily. For the purposes of our investigation, three eggs were randomly selected from each batch. Each of these 3 eggs is treated separately. The albumen and yolk of each egg were separated and 03 samples of each matrix were removed, weighed and analyzed until eggs no longer contain detectable residues of OTC.

Analytical method: The determination of OTC residues in eggs was done using general microbiological methods as they are described in the literature [1] to tissues [6] or the egg [3,5].

Sample preparation: Each array (albumen and yolk) was diluted separately in 1 / 3 in a 1 per cent phosphate buffer at pH 4.5 ± 0.1 (Table 1) and homogenized using a Stovall mixer. The samples were kept in a freezer at (-18 ° C) to their treatment. For the yolk improved the limit of quantification was obtained by centrifugation after freezing the yolk mixture - buffer 4000 rpm for 15 minutes. Heat treatment of the endosperm was not realized because of the test- germ used (Bacillus cereus) for determination of tetracycline resistance to inhibitory substances present in the endosperm diluted (acidic pH).

Table 1

Values of pH of the albumen and yolk diluted in phosphate buffer

Matrix	phosphate buffer pH 4.5 ± 0.1
Albumen (pH = 7.9) 6.4	6.4
Yolk (pH = 6.4) 5.9	5.9

Petri dish Preparation: After inoculation of an agar medium (Antibiotic 8 Difco agar) with a spore suspension of Bacillus cereus ATCC 11778 with a final concentration of 103 organisms /ml.17 bowls (8 mm diameter) were dug on the agar, 08 bowls were filled with 0.2 ml of sample solution at different concentrations, the 08 other bowls are reserved to the reference range and a bowl to witness.

The standard curve: curves standards were made by adding to the albumen and yolk indicator light (test taken 10 g) 1 ml of increasing concentration of OTC (SIGMA) diluted to 1 /3 by adding 19 ml of phosphate buffer 1 per cent at pH 4.5 (Table 1) and mixing, the solutions obtained correspond to different points of range or 4 concentrations of the reference curve (1.2,0.6, 0.3 and 0.15 µg / g) for albumen (Table 4, Figure 1) and 4 levels (2.4,1.2, 0.6 and 0.3 µg / g) for yolk (Table 5, Figure 2). Petri dish (120 x 120 mm) are incubated for 18 hours at 30 ° C. The reading of the diameter of inhibition zones was made using a ruler and a magnifying and illuminating glass.

RESULTS AND DISCUSSION

Various digital data were collected daily and here are some "raw data" (Table 2).

Table 2

Diameters of inhibition zones (mm) obtained during treatment

Time per Day			DURING THE TREATMENT				
			1	2	3	4	5
Egg 1	SAM 1	YOL	UND	11,59	11,78	13,91	12,93
		ALB	UND	11,91	10,77	12,61	10,43
	SAM 2	YOL	UND	12,11	11,26	11,84	12,36
		ALB	11,53	10,63	13,36	11,15	12,93
	SAM 3	YOL	UND	10,57	12,62	13,39	13,86
		ALB	11,94	11,12	13,84	13,11	13,40
Egg 2	SAM 1	YOL	UND	11,59	13,42	14,22	14,74
		ALB	11,24	10,08	13,03	12,32	12,61
	SAM 2	YOL	UND	11,15	11,42	13,73	12,54
		ALB	9,94	12,50	11,52	10,99	11,15
	SAM 3	YOL	UND	10,57	12,92	11,07	14,18
		ALB	11,68	12,96	13,54	11,50	13,11
Egg 3	SAM 1	YOL	UND	UND	13,72	12,21	15,07
		ALB	10,98	12,19	12,73	10,43	12,32
	SAM 2	YOL	UND	11,31	13,24	11,67	14,54
		ALB	9,80	10,78	11,36	12,93	10,99
	SAM 3	YOL	UND	11,79	10,68	13,08	11,72
		ALB	10,25	12,67	11,88	13,40	11,50

Table 3

Diameters of inhibition zones (mm) obtained after treatment

Temps per day			1	2	3	4	5	6	7	8	9	10
Egg 1	SAM 1	YOL	13,05	12,81	14,04	11,90	11,66	11,41	10,15	9,5	ND	ND
		ALB	10,08	UND	11,14	11,42	11,14	9,8	9,51	UND	ND	ND
	SAM 2	YOL	12,47	12,24	11,95	11,38	11,15	10,9	10,12	9,64	ND	ND
		ALB	12,50	11,42	9,85	UND	10,09	11,15	UND	9,33	ND	ND
	SAM 3	YOL	13,98	13,72	13,51	12,75	12,50	10,66	9,9	9,5	ND	ND
		ALB	12,96	11,83	11,57	11,83	10,45	UND	10,83	UND	ND	ND
Egg 2	SAM 1	YOL	14,87	14,60	14,36	13,56	12,29	10,25	9,51	UND	ND	ND
		ALB	12,19	11,14	10,88	11,14	9,84	9,73	UND	UND	ND	ND
	SAM 2	YOL	12,65	12,42	13,86	11,54	11,31	10,44	10,23	9,8	ND	ND
		ALB	10,78	9,85	9,71	9,85	UND	9,8	9,72	UND	ND	ND
	SAM 3	YOL	14,31	14,05	11,17	13,05	12,79	9,35	10,88	UND	ND	ND
		ALB	12,67	11,57	10,16	11,57	10,22	10,65	UND	9,15	ND	ND
Egg3	SAM 1	YOL	15,20	14,92	12,32	13,87	12,39	11,41	10,6	UND	ND	ND
		ALB	11,91	10,88	UND	10,88	UND	10,42	10,11	UND	ND	ND
	SAM 2	YOL	14,67	14,40	11,78	13,38	13,11	10,5	UND	9,56	ND	ND
		ALB	10,63	9,71	11,42	9,71	9,61	UND	10,34	UND	ND	ND
	SAM 3	YOL	11,83	11,61	13,20	10,79	10,57	10,02	9,31	9,34	ND	ND
		ALB	11,12	10,16	11,83	10,16	11,57	10,02	UND	UND	ND	ND

Legend: ALB: albumen, ND : not done, SAMP : sample, UND : Undetectable, YOL : yolk.

For a dose equal to 100 mg / kg / day administered for 5 days orally using an esophageal probe, residues of OTC were detected in the albumen and yolk during treatment and 6 days in albumen and yolk in 7 days after the end of treatment (Table 6, Figure 3). We notice that residues of OTC were present longer in the yolk than in

albumen, but their appearance is faster in the latter (24 hours for 48 hours endosperm cons for the yolk). Indeed, if the residues reach the level of the genital tract, they can permeate the egg albumen in training, which will probably be yellow "protected" from contamination by the membrane layer. It was not until ovulation the oocyte "the most mature, contaminated the ovary, to observe traces of antibiotics in the latter [10]. The study by Roudaut et al. (1987) at a dose that we used showed that the residues are detectable in both the endosperm during treatment and in the yolk after the treatment. Peak concentrations were obtained at day 3 after initiation of treatment for albumen (0.18 µg / g), yolk (0.53 µg / g) and whole egg (0.25 µg / g) 1 day after the end of treatment.

Our study in the same dose (100 mg / kg), maximum concentrations are obtained (0.26 µg / g) in the albumen at day 3 and during treatment (0.58 µg / g) in the yolk to Day 1 after treatment in a limit of quantification respectively (0.08 µg/ g) and (0.27 µg/ g) and maximum concentration in the egg is (0.33 µg/ g) from the last day of treatment. In comparison, the results we obtained are similar to those obtained by Roudaut et al (1987).

Table 4

Diameters of inhibition zones obtained with the concentrations of the standard curve of OTC in the albumen

Concentration (µg/g)	**Diameter of the zones of the inhibition (mm)**					Average
1.2	16.89	17.96	17.5	18.15	17.45	17.59
0.6	14.8	14.98	15.22	15.4	15.1	15.10
0.3	12.2	12.5	13.25	13.33	12.8	12.82
0.15	10.3	11	11.57	11.72	11.5	11.22

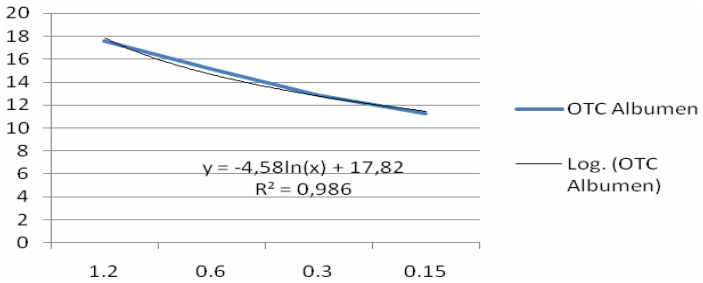

Fig. 1. Standard curve of OTC in the albumen

Table 5

Diameters of inhibition zones obtained with the concentrations of the standard curve of OTC in the Yolk

Concentration (µg/g)	**Diameters of the inhibition zones (mm)**					**average**
2.4	23.33	22.05	23.16	21.5	23.8	22.77
1.2	17.8	18	17.35	17.3	17.9	17.67
0.6	14.4	14.35	14.57	14.7	14.2	14.44
0.3	9.5	9.33	9.65	9.69	9.38	9.51

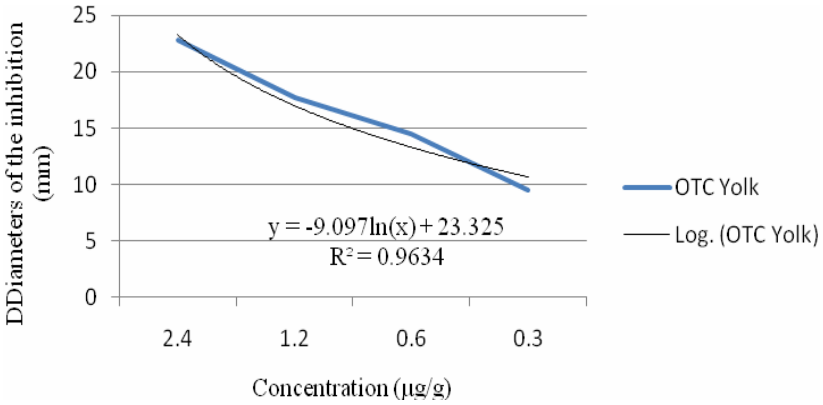

Fig. 2. Standard curve of OTC in the yolk

Table 6

Residus of l'OTC (µg/g) in albumen, yolk and whole egg during and after oral voice administration (100 mg/kg) during 5 days.

	During treatment					After the end of the treatment								
Constituant	1	2	3	4	5	1	2	3	4	5	6	7	8	9
Albumen	0.14	0.20	0.26	0.22	0.22	0.20	0.13	0.13	0.13	0.10	0.09	<0.08	<0.08	ND
Ecart-type	0.016	0.028	0.037	0.033	0.033	0.028	0.01	0.013	0.013	0.031	0.018	UND	UND	UND
Yolk	UND	0.35	0.47	0.51	0.57	0.58	0.56	0.51	0.48	0.44	0.35	0.29	<0.27	ND
Ecart-type	UND	0.071	0.034	0.038	0.044	0.046	0.04	0.038	0.034	0.015	0.027	0.066	UND	UND
Whole egg	0.10	0.24	0.32	0.30	0.33	0.31	0.27	0.24	0.23	0.20	0.18	0.13	UND	ND
Ecart-type	0.011	0.021	0.02	0.019	0.018	0.015	0.02	0.017	0.017	0.017	0.007	0.018	0.032	UND

Legend : ND : not done, UND : undetectable

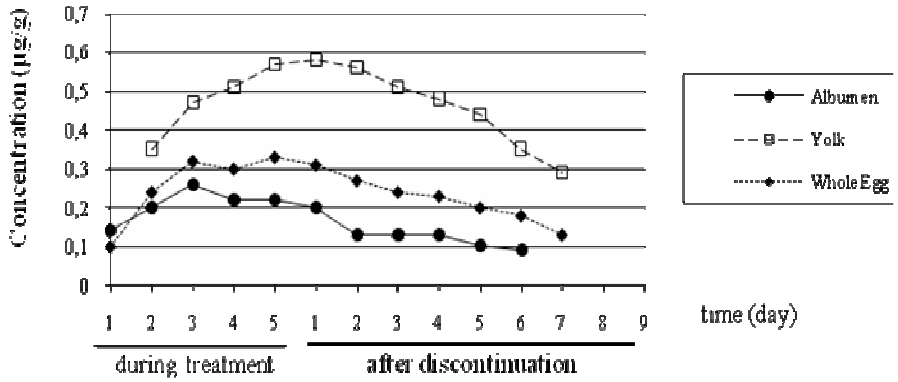

Fig. 3. Evolution of the concentration of OTC (µg/g) in the alumen yolk and whole egg during and after treatment administered orally (esophageal probe: 100 mg / kg / day for 5 days).

Table 7

Waiting time proposed

Constituants of the egg	Administration of l'OTC through oral voice 100 mg/kg/j during 5 days	
	Résidus décelables	Waiting time
Albumen	+ (6 days)	6 days
yolk	+ (7days)	7 days
Whole egg	+ (1 day)	1day

Whole egg : tolerance proposed 0,3 µg/g.

CONCLUSIONS

Our study provides an insight into the real risks of contamination of components of the egg by the OTC in the laying hen. Farmers, in Algeria, must resort to better manage the use of antibiotics, maintenance of a health and breeding records to comply with the rules and waiting times. Improvements are possible through control over all groups' production. Therefore, it is possible to detect farming practices at risk and ensure the quality of eggs supplied to customers (agricultural food industries and pharmacies). It is also necessary to introduce national regulations on the standards of tolerance for each antibiotic in the whole egg for human consumption.

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