

## Residues of Ampicillin in Eggs Following Oral Administration

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**Abstract:** residues in sodium ampicillin (AMP) in eggs of laying hens were determined by a microbiological method of diffusion in agar after oral therapy with an esophageal probe with a dose of 150 mg / kg / day for 5 days. The limit of quantification is 0.02 µg / g in albumen and yolk. The duration of the elimination of AMP in the albumen and yolk is 2 days after stopping the treatment.

**Key words:** Excretion, Residues, Ampicillin Sodium, eggs.

### INTRODUCTION

The harm of some diseases of microbial origin and superinfection in microbial component fully justifies the wide use of antibiotics in laying hens. The conditions of factory farming and the concentration of animals caused by a viable colony high and increased virulence of microorganisms and increases epidemiology. The antibiotic also 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 human health may be of concern, either because of toxic effects of antibiotics, either because of the risk of antibiotic-resistant germs selection [8]. This investigative work aims to help find possible residue of AMP sodium in albumin and yolk of the egg after oral administration with an esophageal probe.

### MATERIAL AND METHODS

**Breeding conditions:** The work has been achieved in the the Regional Veterinary laboratory of Tizi Ouzou on 20 laying hens bred ISABROWN, with an average weight of 1.9 kg, divided into 2 groups (experimental and control), in an animal 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:** AMP sodium was administered daily at the same time, using an esophageal probe with a dose of 150 mg / kg / day for 5 days. The eggs of each batch of chickens were collected daily. For the purposes of the study, three eggs were randomly selected from each batch. Each of these three eggs are 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 AMP sodium.

**Analytical Methods:** Measurement of AMP residues in eggs was done using general microbiological methods described in the literature [2] to tissues [6] or the egg [3,5,1].

**Sample preparation:** Each array (albumen and yolk) was diluted separately in third in a phosphate buffer at pH 6 ± 0.1 percent (Table 1) and homogenized using a Sorvall 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 5000 rpm for 15 minutes. The heat treatment of the mixture albumen - buffer was performed at 70 ° C for 15 minutes.

**Petri dish preparation:** After inoculation of an agar medium (Antibiotic 8 Difco agar) with a suspension of *Sarcina lutea* ATCC 9341 [4,5,2] with a final concentration of  $10^3$  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:** standard curves were made by adding to the albumen and yolk indicator light (test taken 10 g) 1 ml of sodium increasing concentration of AMP (Sigma) diluted to 1 / 3 by adding 19 ml of a phosphate buffer at pH  $6 \pm 0.1$  percent (Table 1) and mixing, the solutions obtained correspond to different points of range or 4 concentrations of the reference range (0.16,0.08, 0.04 and 0.02  $\mu\text{g} / \text{g}$ ) for albumen (Table 4, Figure 1) and four concentrations (0.16,0.08, 0.04 and 0.02  $\text{mg} / \text{g}$ ) for the yolk (Table 5, Figure 2). Petri dishes (120 x 120 mm) are incubated for 18 hours at 37 °C. The reading of the diameter of inhibition zones was made using a ruler and a magnifying glass illuminating

Table 1

Values of pH of the albumen and yolk diluted in phosphate buffer at pH6.

Matrix	phosphate buffer pH $6 \pm 0.1$
Albumen (pH = 7.9)	6.6
Yolk (pH = 6.4)	6.2

## RESULTS AND DISCUSSION

Lot of digital data were collected daily and are "raw data" (Table 2.3). For a dose equal to 150 mg / kg / day administered for 5 days orally using an esophageal probe, AMP sodium residues are detected on the first day in the albumen and the second day in yolk during treatment. AMP sodium residues persist until the second day in the yolk and they will no longer be detected in the albumen after the end of treatment (Table 6, Figure 3). Residues of the AMP longer are present in the yolk than in the albumen (two days), but their onset is faster in the latter (24 hours for 48 hours albumen cons to 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 sac. It was not until ovulation the oocyte "the most mature, contaminated the ovary, to observe traces of antibiotics in the latter [8]. The results are consistent with the mechanisms involved at the time of egg formation in the ovary and with the respective sites of yolk synthesis (liver) and albumen (oviduct) [4]. The study by Roudaut (1987) at a dose (150 mg / kg) showed that residues were detectable in the albumen as well as in the yolk during and even after the treatment period. Maximum concentrations were obtained at 4th day after beginning treatment for albumen (0,008  $\mu\text{g} / \text{g}$ ), yolk (0,028  $\mu\text{g} / \text{g}$ ) and whole egg (0,015  $\mu\text{g}/\text{g}$ ) and then decreased rapidly from the last day of treatment.

Our study of the same dose such as (150 mg / kg) showed maximum concentrations in the albumen with the 2nd day (0,038  $\mu\text{g} / \text{g}$ ) and 4th day in the yolk with (0,041 $\mu\text{g} / \text{g}$ ) and egg whole day with the 3rd (0,037  $\mu\text{g} / \text{g}$ ) and then they decrease rapidly from the 2nd day after discontinuation of treatment and the yolk from the last day of treatment for the albumen and whole egg. A comparison of the results reveals that the highest concentrations obtained in

our experiments are five times higher in the albumen and almost double for the yolk. The method used orally using an esophageal probe could explain this discrepancy results.

Table 2

Diameters of inhibition zones (mm) obtained during the treatment of 150 mg / kg of Ampicillin orally.

Time in days			DURING TREATMENT				
			1	2	3	4	5
Egg 1	Samp .1	YOL	UND	UND	12.47	11.37	9.46
		ALB.	UND	10.82	9.68	UND	10.90
	Samp.2	YOL	UND	9.64	12.42	10.65	9.97
		ALB.	10.66	11.11	UND	10.92	UND
	Samp .3	YOL	UND	9.89	12.15	11.93	10.23
		ALB.	UND	11.06	9.44	UND	UND
Egg 2	Samp .1	YOL	UND	10.30	11.94	12.59	10.65
		ALB.	10.86	10.63	10.09	11.12	10.71
	Samp .2	YOL	UND	10.81	11.68	10.92	11.18
		ALB.	9.54	10.40	10.58	9.78	10.47
	Samp 3	YOL	UND	11.05	11.13	12.42	11.43
		ALB.	UND	9.91	10.82	UND	9.98
Egg 3	Samp .1	YOL	UND	11.50	10.42	12.64	11.89
		ALB.	10.43	9.52	11.02	10.69	9.58
	Samp .2	YOL	UND	UND	9.88	12.20	11.94
		ALB.	UND	UND	11.26	9.53	UND
	Samp .3	YOL.	UND	11.25	10.69	10.10	11.63
		ALB.	9.94	UND	UND	10.18	UND

Legend: Samp: Sample, YOL: Yolk, ALB: Albumen, UND: undetectable.

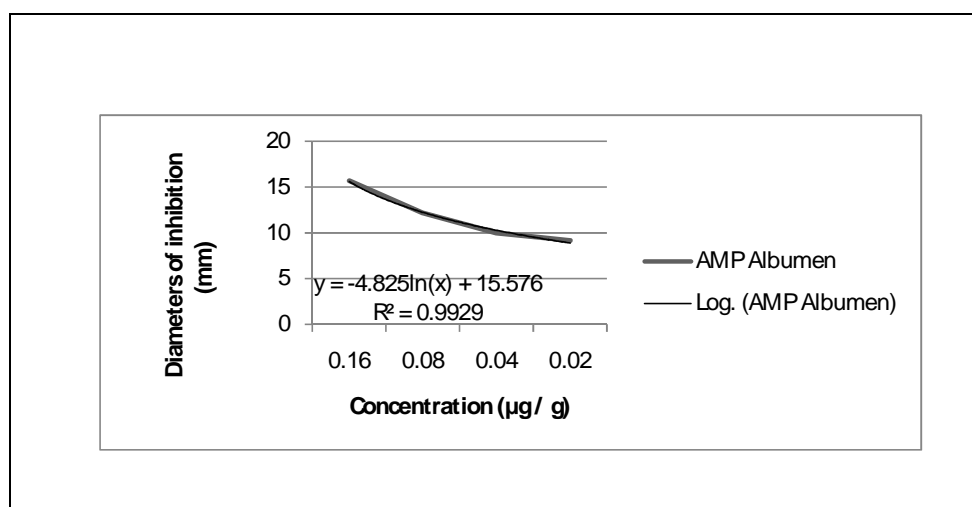


Figure 1: Standard curve of AMP in the albumen

Table 3

Diameters of inhibition zones (mm) obtained after treatment of 150 mg / kg of ampicillin orally

Time in days			When stopping the treatment.				Treatment					
			1	2	3	4	5	6	7	8	9	10
Egg 1	Samp1	YOL	11.59	10.30	UND	UND	UND	UND	ND	ND	ND	ND
		ALB.	UND	UND	UND	UND	UND	UND	ND	ND	ND	ND
	Samp.2	YOL .	11.54	9.64	UND	UND	UND	UND	ND	ND	ND	ND
		ALB.	UND	UND	UND	UND	UND	UND	ND	ND	ND	ND
	Samp.3	YOL .	11.29	10.81	UND	UND	UND	UND	ND	ND	ND	ND
		ALB.	UND	9.50	UND	UND	UND	UND	ND	ND	ND	ND
Egg 2	Samp 1	YOL .	11.09	11.50	UND	UND	UND	UND	ND	ND	ND	ND
		ALB.	UND	UND	UND	UND	UND	UND	ND	ND	ND	ND
	Samp.2	YOL .	10.85	UND	9.72	UND	UND	UND	ND	ND	ND	ND
		ALB.	UND	UND	UND	UND	UND	UND	ND	ND	ND	ND
	Samp.3	YOL .	10.34	1.25	9.93	UND	UND	UND	ND	ND	ND	ND
		ALB.	UND	UND	UND	UND	UND	UND	ND	ND	ND	ND
Egg 3	Samp.1	YOL .	9.68	11.54	10.33	UND	UND	UND	ND	ND	ND	ND
		ALB.	9.58	UND	UND	UND	UND	UND	ND	ND	ND	ND
	Samp.2	YOL .	UND	11.05	10.37	UND	UND	UND	ND	ND	ND	ND
		ALB.	9.79	UND	UND	UND	UND	UND	ND	ND	ND	ND
	Samp.3	YOL .	9.93	UND	10.11	UND	UND	UND	ND	ND	ND	ND
		ALB.	9.83	UND	UND	UND	UND	UND	ND	ND	ND	ND

Legend: Sample, YOL: YOLK, ALB: Albumen, UND: Not detectable, UND: undetectable.

Table 4

Diameters of inhibition zones (mm) obtained with the concentrations of the standard curve of the AMP in the albumen

Concentration ( $\mu\text{g/g}$ )	Diameters of inhibition zones (mm)				Middle
0.16	15.00	15.50	16.17	16.00	15.67
0.08	12.40	12.07	12.40	11.80	12.17
0.04	10.17	9.90	10.06	9.74	9.97
0.02	9.21	9.20	9.11	9.11	9.16

Table 5

Diameters of inhibition zones (mm) obtained with the concentrations of the standard range of AMP in the yolk.

Concentration ( $\mu\text{g/g}$ )	Diameters of inhibition zones (mm)				Middle
0.16	16.00	16.40	17.89	15.30	16.40
0.08	14.00	14.47	13.74	15.67	14.47
0.04	11.40	11.50	11.30	11.80	11.50
0.02	9.31	9.23	9.22	9.15	9.23

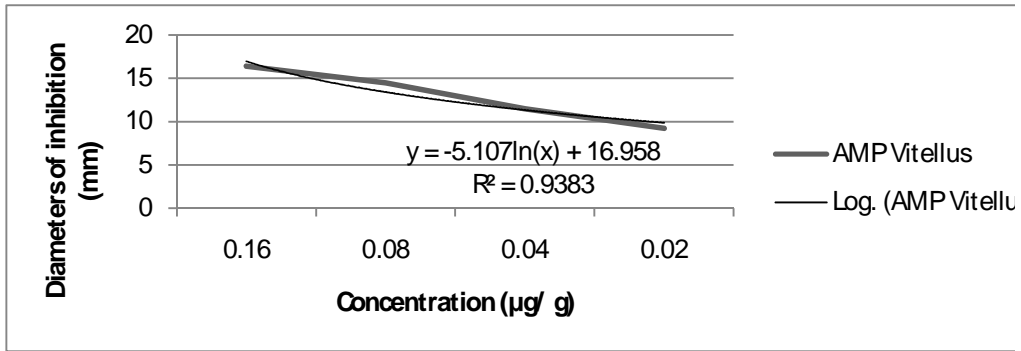


Figure 2: Standard curve of AMP in the yolk

Table 6

Residues of the AMP (mg / g) in albumen, yolk and whole egg during and after oral treatment (150 mg / kg) for 5 days

Time in days	DURING TREATMENT					AFTER THE END OF TREATMENT				
	1	2	3	4	5	1	2	3	4	5
ALBUMEN	0.024	0.038	0.036	0.031	0.025	<0.02	<0.02	UND	UND	UND
Standard deviation	0.006	0.026	0.015	0.011	0.019					
YOLK	UND	0.024	0.038	0.041	0.033	0.028	0.025	<0.02	UND	UND
Standard deviation		0.008	0.011	0.004	0.009	0.011	0.001			
WHOLE EGG	0.017	0.034	0.037	0.033	0.028	0.017	0.010	UND	UND	UND
Standard deviation	0.004	0.017	0.009	0.009	0.013	0.009	0.004			

Legend: UND; Not detected, albumen and yolk: with a sensitivity limit of 0.02 µg / g.

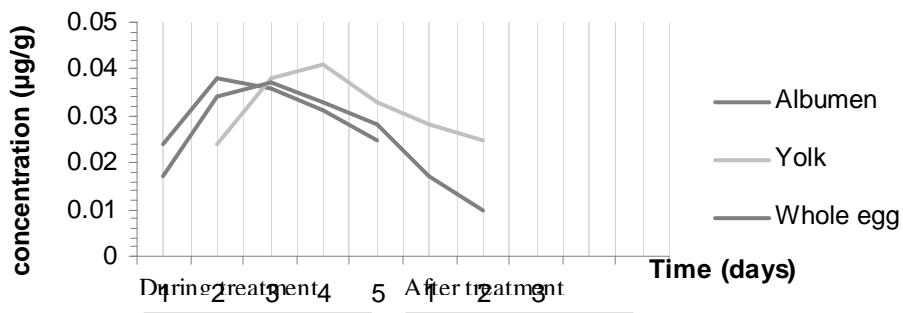


Figure 3: Evolution of the concentration of AMP (µg / g) in albumen, yolk and whole egg during and after treatment administered orally (oesophageal probe: 150 mg / kg / day for 5 days).

Table 7

## The proposed withdrawal

Constituents of the egg	Administration of AMP through oral voice 150 mg / kg / day for 5 days	
	Residues detectable	waiting time
Albumen	+ (0 day)	(0 day)
Yolk	+ (2 days)	+ (2 days)
Whole egg	+ (2 days)	+ (2 days)

whole egg: tolerance proposed 0.01 µg / g.

## CONCLUSIONS

This study shows that there are real risks of contamination of the egg constituents by residues of the AMP sodium in the laying hen. We must therefore define a delay before delivery of each constituent of the egg to ensure product quality provided to customers (industrial food processing and pharmacies). It appears so urgent that safety standards can be defined for each antibiotic used on the national level, so that the farmer can continue to benefit from this therapy without the consumer's health at risk is unacceptable.

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