

## Study of Doxycycline Efficacy in Broilers' Colibacillosis

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### Abstract

The aim of study is a therapeutic assessment of doxycycline's hyclate efficacy, a wide spectrum antibiotic (efficient in majority of bacterial Gram-positive and Gram-negative infections and mycoplasmas, frequently isolated in poultry) in a colisepticemia outbreak in a 3,000 chicken farm. Disease started at the broiler's age of 20 days and mortality rate reached 32.5%. The behaviour to antibiotics of isolated bacterial strains was tested and antibiogram (Sanofi) was used, doxycycline revealing a very good antibiotic activity. The treatments (with the dose of 10 mg x kg.b.w.<sup>-1</sup>) were done individually for five days, clinical evolution being observed until day ten of the experiment. Analyzing evolutions after treatments it was observed that the mortality diminished progressively from 14.2% in the first day, to 8.3% - 5.9% - 2.5% - 1.7% and respectively 0.8% in the sixth treatments day, in days seven, eight, nine, and ten of the study not being registered any mortality cases. In our opinion the favorable clinical evolution was recorded after the third treatment day, at the end of experiment from 120 remaining live 80 chickens (representing 66.7%) proving the fact that doxycycline was efficient, confirmed also by the average daily weight gain for the remaining live chickens.

**Keywords:** broiler chickens, colibacillosis, doxycycline, efficacy

### 1. Introduction

The aim of present study is a therapeutic assessment of doxycycline's hyclate efficacy in broilers in a colisepticemia outbreak.

Doxycycline is a soluble powder suitable for administrations in drinking water to poultry.

### 2. Materials and methods

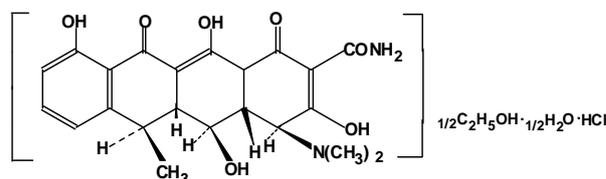
*Doxycycline hyclate* is a structural isomer, the hemiethanolic hydrochlorate - hemihydrate of (4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-dimethylamino-1,4,4*a*,5,5*a*,6,11,12a-octahydro-3,5,10,12,12*a* pentahydroxi-6-metil-1,11-dioxonaftacen-2-carboxamide (IUPAC), who meets the European reference standards (*Ph.Eur. III 1997:0272*) and

respect C.E.E. Regulation (nr. 2377/90), for M.R.L. (*Maximum Residue Limits*) published in O.J. (*Official Journal of the European Communities*) and also the latest MRL-regulations: Commission Regulation (EU) No 37/2010 of 22 December 2009, (*OJ L15/1* of 20.1.2010) [1, 2].

*Molecular formula:* C<sub>22</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>8</sub>, 1/2C<sub>2</sub>H<sub>6</sub>O, 1/2 H<sub>2</sub>O

*Molecular mass:* M: 512,9 and

*Structural formula:*



**Figure 1.** Structural formula of doxycycline hyclate [2]

Doxycycline is an inhibitor of protein synthesis in sensible organisms and it traverse directly through the bilipidic layer of bacterial wall [3, 4, 5].

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Once reached in the bacterial cell, doxycycline is coupling to the 30S ribosomal unit inhibiting the bacterial complex named *tARN-aminoacyl-mARN-ribosomal* who is implied in the translation phase. Doxycycline inhibits the activity of metal proteinase matrix (in mammalians), migration and degranulation of neutrophilic mediated tissues, oxygen and arahidonic acid synthesis and respectively of prostaglandins [4, 5, 6, 7]. Also act as NO synthesis inhibitor and of endogenic synthetase (nitric oxide inductor) [4]. The coupling to ribosomes will assure the transport in the bacterial cell's interior, being considered a biphasic process, (the plasmatic absorption being followed by active transport processes) [4, 5, 6, 7].

Additionally, effects on leukocytes (chemotaxis and phagocytary inhibition) are also registered.

*Therapeutic indications:*

Doxycycline has a wide spectrum being efficient in majority of bacterial Gram-positive and Gram-negative infections and mycoplasmas, frequently isolated in poultry (see Table 1).

The majority of these bacteria are often isolated and presented in veterinary literature, especially in the respiratory, genital and digestive tract, in numerous species, including poultry [8, 9, 10, 11, 12, 13].

In table 2 are presented data about the poultry isolates' sensibility.

**Table 1.** Spectrum and sensibility of microorganisms to doxycycline hyclate [after: 8, 9, 10, 11, 12, 13]

Microorganism	Great sensitive (CMI <sub>90</sub> <4µg/ml)	Medium sensitive (CMI <sub>90</sub> <16µg/ml)	Resistant species (CMI <sub>90</sub> >16µg/ml)
	<i>Haemophilus</i>	<i>Klebsiella</i>	<i>Proteus</i>
	<i>Neisseria</i>	<i>Escherichia</i>	<i>Pseudomonas</i>
	<i>Brucella</i>	<i>Salmonella</i>	
Gram negative	<i>Campilobacter</i>	<i>Enterobacter</i>	
	<i>Moraxella</i>	<i>Serratia</i>	
	<i>Yersinia</i>	<i>Shigella</i>	
	<i>Pasteurella</i>	<i>Bordetella</i>	
	<i>Vibrio</i>		
Gram positive	<i>Staphylococcus</i>	<i>Streptococcus</i>	
	<i>Corynebacterium</i>		-
	<i>Bacillus</i>		
	<i>Listeria</i>		
Anaerobic bacterias	<i>Fusobacterium</i>	<i>Bacterioides</i>	
	<i>Actinomyces</i>	<i>Clostridium</i>	-
	<i>Propionibacterium</i>	<i>Lactobacillus</i>	
	<i>Spirocheta</i>		
	<i>Mycoplasma</i>		
Other microorganisms	<i>Rickettsia</i>		
	<i>Chlamidia</i>	-	-
	<i>Ureaplasma</i>		
	<i>Erlchia</i>		
	<i>Anaplasma</i>		

**Table 2.** Sensibility to doxycycline of poultry isolates

<i>In vitro</i>	Sensibility to doxycycline	Authors
<u>Chicken isolates :</u>	To numbered genus:	Ramasastry, 1991 [10]
<i>E.coli</i> ,	25% sensibility	Bousquet, 1994 [8]
<i>Staphylococcus</i> ,		
<i>Streptococcus</i> ,		
<i>Pasteurella</i> ,		
<i>Enterobacter</i> ,		
<i>Pseudomonas</i> ,		
<i>Bacillus</i> , <i>Klebsiella</i>		
<i>Campylobacter coli</i>	100%	*** [13]
<i>Campilobacter jejuni</i>	98%	
<i>E. coli 65</i>	Low sensibility	Prasad, 1997 [9]
<i>Salmonella gallinarum</i>	43%	
<i>Salmonella enteritidis</i>	98%	Ramasastry, 1991 [10]
<u>Tukey isolates:</u>		
<i>Chlamidia psittaci</i>	100%	*** [13]

General dose is: 10 mg doxycycline x kg.bw<sup>-1</sup>, oral way in fodder or water [1, 6, 14, 15].

Before treatments chickens will be deprived by water, introducing the fluid diet [1, 15].

Doxicycline is diluted in a water quantity required for 3 hours and in the necessary of water amount calculation ambient and body temperature of animals must be considered [1, 15].

*Research methodology:*

- Disease cases identifying and lots' setting
- Etiologic diagnosis
- Antibioqram (difusometric method)
- Product administration after recommendations
- Clinical monitoring after treatments
- Weight gain evolution monitoring

*The animals:*

The product was clinically tested in three week old Cobb hybrids in a 3,000 heads farm, where broilers are grown on the ground.

Epidemiological investigation revealed the presence of favorising factors like combined zoo hygiene deficiencies, moisture, agglomeration, temperature variations and thermal discomfort.

Under these conditions, frequent enteric episodes were recorded.

Disease started at the broiler's age of 20 days and mortality rate reached at 32.5%.

Lack of appetite and polydipsia, were additional signs that indicated colisepticemia.

Disease was confirmed by morphopathologic and bacteriological examination.

Health and bio-parameters were followed through:

- clinical examination: appetite, temperature, specific consumption (fodder and water), mobility, enteric and respiratory signs.
- morphopathologic exam: necropsy of all dead chickens.

In the hall was built a 4 x 5m pen, where 120 chickens, all with clinical colisepticemia signs, were randomly placed for the experiment.

A group of 12 chickens constituted the Control lot who do not received any treatment, only clinic evolution was monitorized.

The chickens were fed ad libitum with a balanced and of quality mixed fodder (CC 1115 <sup>(Vivabio)</sup>).

Watering was done at the discretion.

*Methods:*

Disease presence and cases frequency was confirmed after the morphopathological and bacteriological examination.

Bacteriological examinations revealed the presence of numerous strains of *Escherichia coli* (11 strains).

The behaviour to antibiotics of isolated bacterial strains was tested and antibiogram <sup>(Sanofi)</sup> was used. The used micro tablets were: Colistin sulphate, Neomycin, Amoxiklav, Enrofloxacin, Norfloxacin, Erythromycin, Spectinomycin and Doxicycline.

The proportion of resistant strains to multiple antibiotics was variable, showing high efficacy of Colistin sulphate, isolates of *E. coli* being also very sensitive to doxicycline, confirming other results from literature [9, 16].

Treatments duration was of five days and monitoring period was finished ten days after the starting of treatments.

### 3. Results and discussion

Analyzing evolution after first day of treatments it was observed that the mortality had quite great values (14.2%), comparatively to the mortality existing in the hall.

After the second and third day of therapy, mortality levels have maintained to great values but with a clear decreasing trend (from 8.3% to 5.9%). In the coming two days, mortality levels declined significantly (between 2.5% and 1.7%).

Mortality recorded after six days of treatment period showed low values (0.8%).

In the day: seven, eight, nine and ten of the study have not registered any mortality in chickens.

In our opinion the favorable clinical evolution was recorded after the third day of treatment, (in the seventh day mortality being totally stopped), at the end of experiment from 120 remaining live 80 chickens, (representing 66.7%). Similar results can be founded in literature [15, 17, 18].

The general health condition improved and after 5 days of treatment when the clinical signs have entirely disappeared.

In the untreated Control group, all chickens have died until fourth day.

The good results obtained confirm the therapeutic efficacy of the studied product in an outbreak of colibacillosis, although the losses recorded, confirming the results of antibiograms [4, 9, 13].

To all experiment duration dead chickens were morphopathologically studied being revealed all colisepticemia characteristic known lesions: spleen hypertrophy, bleedings, perihepatitis, pericarditis, aerosaculite.

Although doxycycline was highly effective in colisepticemia, irreversible damages leading to death installed in chickens.

This can be an explanation why mortality remained relatively high during the first three days of treatment.

It should be noted also that chickens treated with doxycycline hyclate were presenting different evolutionary stages of colibacillosis symptoms.

As is known, chickens with advanced forms of disease could not be recovered even if the drug administered was bacteriologically effective [3]. This was confirmed by the results of bacteriological examination, from the chickens undergoing treatment we isolated strains of *E. coli*.

In the coming days mortality was significantly reduced and the result of bacteriological tests was negative.

Evolution of colisepticemia losses in studied chicken is reproduced in Figure 2.

After treatments the chickens' clinic and biologic recovery was good, fact proven by the evolution of weight body gain for the living broilers who were weighted (in days; 20, 30, 40 respectively 50) when chicken were sacrificed.

The weight values and specific consumption rates for the living poultry was between Cobb hybrid parameters starting from day 30

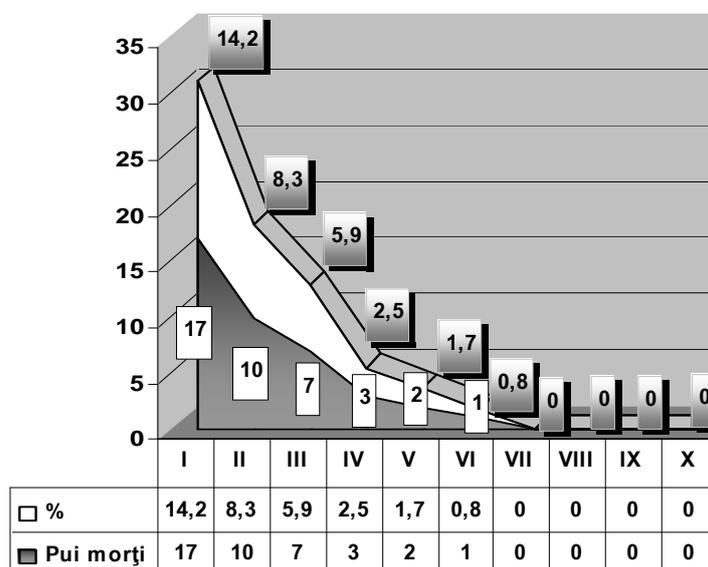


Figure 2. Losses' evolution in the studied group ■ = dead chicken

Table 3. Average daily weight gain, average fodder and water daily consumption / studied period

Lot in day	Total weight/ Lot	Average weight	Average daily body gain / period (g)	Average daily fodder consumption (g)	Average daily water consumption (ml)
Day 20 120 heads	73800 g	655 g	30,75	56 (≅ 6,5 kg)	≅ 200
Day 30 40 losses	75280 g	941 g	24,60	110 (8,80 kg)	300
Day 40 80 remained	119600	1495 g	51,40	170 (13,60 kg)	370
Day 50 Slaughtering	174400	2180	68,50	190 (15,20 kg)	450

#### 4. Conclusions

- Doxycycline in dose of 10 mg x kg.b.w.<sup>-1</sup> administered for five days resulted in a complete clinical and bacteriological healing of the broilers with colisepticemia and recovered in day ten.

- In all cases, therapy is preferable, even if the economic parameters are significantly lower than disease-free situations.
- After treatments, the clinical and biological recovery was evidenced also by the evolution of weight gain in the remaining chickens.

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