Safety Study of Doxicycline in Broiler Chickens

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Abstract
Doxicycline is a structural isomer who meets the European reference standard (*Ph.Eur. III 1997:0272*) and respect EU Regulations for M.R.L. The good solubility permits high tissular absorption and distribution after administration in drinking water. The aim was to ascertain the clinical effects and tolerance consecutive to risen doses administration, as a part of safety study for this antibiotic in poultry in the respect of current drug testing methodology. The safety study revealed: good local and general tolerance to therapeutic doses (10 mg x kgb.w.-1) and to x2 dose, diarrhoea in lots which received x3 and x5 the therapeutic dose. Comparatively with the Control lot, haemoleucogram doesn't suffer evident changes the registered values being between the references limits (exception of leucocitary formula). Also it was found an increased creatinin concentration consecutively to x3 and x5 greater doses administration and marked increasing of ASAT level and limited for ALAT's, comparatively to control lot in the case of E_4 lot, but light to reference values. Macro and microscopy revealed for the liver samples: hepatomegaly, diffuse hepatic degenerescence, nuclear heterochromatinization, vacuolar degenerescence; renal changes in nefrons and renal corpuscles, light splenomegaly, caecal sacs distension and brown-yellowish gaseous content to lots E_3 and E_4.

Keywords: *doxicycline, tolerance, broiler chicken, safety study*

1. Introduction

The study proposes the safety evaluation for a new a.u.v. tetracycline, the doxicycline, destined for broiler chickens in water administrations.
The aim of the safety study was to ascertain the clinical effects and tolerance consecutive to risen doses administration, as a part of safety study for this antibiotic in poultry.

2. Materials and methods

Doxicycline hyclate is a structural isomer 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 în 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, 3].
As all tetracycline family representatives, doxicycline is an inhibitor of protein synthesis in sensible organisms and it traverse directly through bilipidic layer of bacterial wall [4, 5, 6].
Metallic ions especially of iron, significantly lowers the efficacy of active substance, consecutively to oral administration due to insoluble compounds appearance (iron chelates).
Good solubilisation permits a high absorption and distribution in tissues and organs after administration in drinking water [7, 8, 9].

The studied compound:

Composition:

Doxicycline hyclate	0,30 g.
Excipients q.s. ad	1,00 g.

Compound's presentation:

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Veterinary use (a.u.v.) powder, crystalline, yellow, hygroscopic, for internal use.
In concentration of 100 ml in 1 litre of drinking water the powder is easy soluble, in methanol is slightly soluble, in ether being insoluble.
The powder is dissolving also in alkaline solutions of hydroxides and carbonates [2, 4].

Target species: broiler chickens and turkeys.

Indications: Wide spectrum antibiotic, efficient in almost all Gram-positive and Gram-negative bacterial infections, in mycoplasmas frequently isolated in poultry, *Riketsia, Chlamydia* and some protozoa [10, 11].
The powder will be diluted in drinking water consumable for three hours. To the calculation of water necessary, medium and corporal temperature will be heed.

Contraindications: do not use in the same time with other antibiotics.

Waiting time: for broiler chickens = 7 days [2];
for meat turkeys =10 days [2].

Warnings: do not use to the treatment of laying hens!

Animals:
The estimation of tolerance and effects consecutive to, two, three and five times growing doses greater that therapeutic was made for five days in five one week old Cobb 500 broiler chickens lots as follows:

- Experimental lot 1 (E_1):
  therapeutic dose = 10 mg x $kgbw^{-1}$ per day,
- Experimental lot 2 (E_2):
  two times the therapeutic dose,
- Experimental lot 3 (E_3): three times the therapeutic dose,
- Experimental lot 4 (E_4):
  five times the therapeutic dose,
- Control lot (C):
  no drug administration, only water.

Administrations were made individually, per os, boilers being kept on ground, feeding and watering being *ab libitum* with quality well balanced fodder in conformity with the age category. The followed parameters were: health status and bio productivity.

Health status was established after the:

Clinic examination of:
- general and local tolerance,
- appetite

Paraclinic examination:
- haemoleucograme, erythrocytes, leucocytes, haemoglobin, haematocrit, leucograme;
- sanguine biochemical exam: total proteins level, albumins, globulins, creatinin, uric acid and enzyme values for: ASAT, ALAT and ALP.
- histologic and morphopathologic examinations

Methods:
Haematologic examination (haemoleucograme) was accomplished with MS-9-VET automatic analyzer and biochemical examination with VET SCREEN semiautomatic analyzer.
To the last day of the experiment the chickens were sacrificed and the necropsy and samples for histology gathering being made.
The tissues (liver, kidney and spleen) were fixed in 80^c alcohol, included in paraffin, sectioned to 5µ and H.E., respectively *Mallory* tri-chromic coloration.

3. Results and discussion

Clinic examination

Health parameters evidenced a very good general and local tolerance for the subjects from E_1 and E_2 lots.
In the case of E_3 lot and especially in E_4 lot diarrhoea was observed diarrhoea but with no other modifications.
Appetite for fodder and water was lightly modified, for E_4 lot (for fodder) and respectively for E_3 and E_4 lots (for water).

Bio productive parameters

Daily medium gain, daily fodder and water consumption are presented in table 1.
Administering of doxicycline powder to (% x lots E_{1+4}/x lot C) determined:
- diminution of daily b.w. medium gain in lots $E_{2,3,4}$ (-21,01%-E_2; -22,82%-E_3; respectively -24,18%-E_4);
- limited diminution of daily fodder consumption (-2,08%-E_2; -2,08%-E_3; respectively -14,26%-E_4);
- diminution of daily water consumption (-1,86% -E_1; -7,34%-E_2; -28,02%-E_3; respectively -28,86%-E_4).

Paraclinic examination

The results for haematological examination are presented in table 2

Table 1. Daily b.w. medium gain, daily fodder consumption and daily water consumption in the studied lots

Lot	Daily b.w. medium gain (g)	Daily fodder consumption (g)	Daily water consumption (ml)
E_1	17.06	34.5	1.6
E_2	13.98	33.78	67.6
E_3	13.66	33.78	52.51
E_4	13.42	29.58	51.9
C	17.7	34.5	72.96

Note: Accepted reference values are:
- Daily body weight gain (for Cobb, hybrid Hubbard broilers): 23,0 g, 16,7 g [12],
- Daily fodder consumption: 21 g, 24-40 g [5], 32,2 g [13].
- Daily water consumption: 76-100 l/1000 chicken [13].

Table 2. Haemoleucograme for the broilers treated with doxicycline

Specification	Arithmetic mean ± Average mean error				
	E_1	E_2	E_3	E_4	C
Erythrocytes (x 10^{12}/l)	2.33 ± 0.09	2.28 ± 0.063	2.24 ± 0.06	2.06 ± 0.076	2.52 ± 0.082
Haematocrit (%)	31.75 ± 0.77	30.8 ± 1.23	29.7 ± 1.10	28.75 ± 0.92	32.3 ± 1.42
Haemoglobin (g/100 ml)	10.75 ± 0.33	10.4 ± 0.24	10.7 ± 0.27	10.9 ± 0.36	10.15 ± 0.17
Leucocytes (x 10^9/l)	19.03 ± 1.6	18.4 ± 1.71	18.58 ± 1.43	19.68 ± 1.22	18.69 ± 0.92
Lymphocytes (%)	92.10 ± 2.42	93.05 ± 3.13	92.85 ± 2.32	92.2 ± 2.84	91.5 ± 1.92
Granulocytes (%)	4.35 ± 0.51	3.45 ± 0.36	3.7 ± 0.45	4.35 ± 0.43	4.6 ± 0.66
Monocites (%)	3.55 ± 0.62	3.5 ± 0.73	3.5 ± 0.48	3.55 ± 0.53	3.55 ± 0.7

Note: accepted reference values (x ± DS) for broilers are [14]:
- *erythrocytes* 2,35 ±0,25;
- *haematocrit* 26 ±4;
- *haemoglobin* 7,3 ±1,3;
- *leucocytes* 26 ±4;
- *lymphocytes* 63 ±10;
- *granulocytes* 35,5 ±7,8;
- *monocites* 6,1.

The administration of doxicycline (% x lots E_{1+4}/ x lot C) determined low amplitude fluctuations for the level of:

*total proteins (+4,16%-E_1; -3,27%-E_2; -15,17%-E_3; -0,29%-E_4),

*albumins (-4,34%-E_1; -13%-E_2; +8,69% -E_3; +13,04%-E_4),

*globulins (+15,9%-E_1; +2,27%-E_2; -13,18%-E_3; -6,81%-E_4), uncorrelated with dosage and in the reference values limits;

- rise of creatinin concentrations after x3 and especially x5 times therapeutic dose (+28,57%-E_3; +42,85%-E_4);
- decrease of uric acid level (-63,12%-E_1; -34,37%-E_2; -50%-E_3; -25,62%-E_4); uncorrelated with dosage and in the reference values limits;
- progressive growth in parallel with dose increasing of GOT (+6,28%- E_1; +6,55%-E_2; +9,28%-E_3; +14,2%-E_4) and pronounced to the five times therapeutic doses,
- ASAT levels increase (+12,12%-E_1; +27,27%-E_2; +36,36%-E_3; +118,18%-E_4);
- ALP levels decrease in lots E_1:-27,02%; E_2:-31,96% and E_3:-12,23% and slight increase to lot E_4:+1,19%.

The pronounced increase of ASAT levels and limited for ALAT in lot E_4, indicated the possible hepatic injury to the individuals that received x5 times the therapeutic dose.

Morphopathological and histopathological investigation

Morphopathological modifications were registered only for the E_2, E_3 and E_4 lots and consisted in:

E_2: light hepatomegaly
growth in volume of the caecal sacs

E_3: light hepatomegaly
low intensity and diffuse zones of hepatic degenerescence
light splenomegaly
intestinal vascular ectasia
caecal sacs distension and gaseous content

E_4: hepatomegaly
extended but diffuse zones of hepatic degenerescence
light splenomegaly
distension of caecal sacs; brown-yellowish gaseous content.

Following of the liver histologic investigation, sections obtained from E_1, and E_2 lots, there were

not observed tissue structural modifications, but for the E_3 and E_4 lots samples it was observed the hepatocite's nuclear heterochromatinization. This can be considered as an outcome of hepatocite's decreased metabolic synthesis and is considered as a defensive stage of the hepatic cells against stress aggression.

In E_3 and E_4 lots samples, vacuolar degenerescence was pointed out (see figure 1), justifying the growth tendency of the specific hepatic enzymes.

The examination of histologic sections through the kidney samples to the E_1 and E_2 not emphasised any significant citohistological changes, nephrocites having a normal aspect with no citoplasmatic vacuoles.

Vascular glomerule and Bowman capsule were maintained them integrity.

In the case of E_3 and E_4 lots the registered alterations were found in nefrons and especially renal corpuscles (see figure 2).

These aspects revealing affecting of the filtering, resorption and renal excretion processes with heavy consequences on general organism's metabolism.

Finally, the microscopic examination of spleen in the E_1, E_2, E_3 lots samples does not shown evident morphologic changes, the lymphoid follicles and them perivascular lymphoid case, the red pulp with its vascular netting and the cellular belts does not presented alterative changes.

Only in the case of E_4 lot samples was observed the red pulps' extension with evident vascular ectasia (see figure 3).

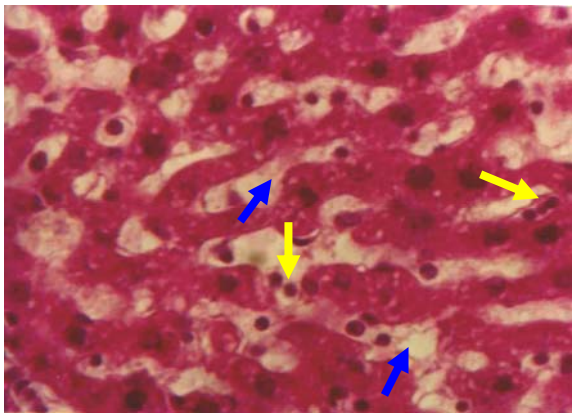

Figure 1. Histologic section through hepatic tissue from E4 lot's samples
Nuclear heterochromatinization (picnotic nucleous-necrobiosys (yellow). Hepatocite's vacuolar degenerescence (blue)

The haematogenous marrow microscopy of all samples does not reveal any morphopathologic alteration.

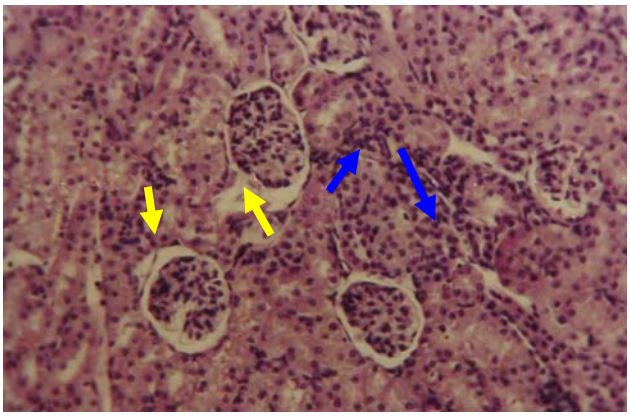

Figure 2. Histologic section through kidney tissue samples from the E4 lot's samples
Renal corpuscles degenerescence, with the Bowman's capsule altering (yellow). Red marrow increasing with erythrocyte's abundance (blue)

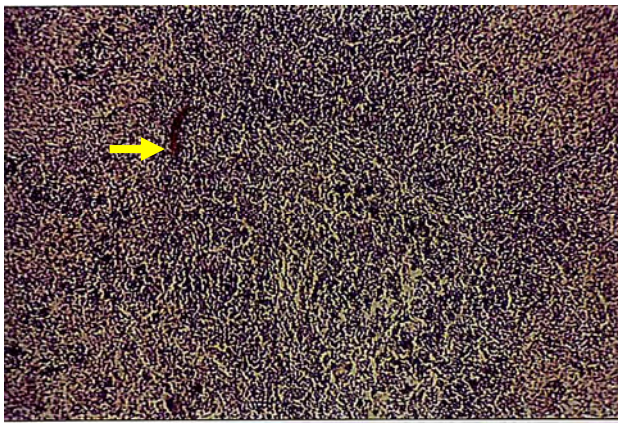

Figure 2. Histologic section through spleen in E4 lot samples
Spleen's vascular ectasia

4. Conclusions

The doxicycline safety study revealed the following aspects:

- Good local and general tolerance to the therapeutic dose (10 mg x kgb.w.$^{-1}$) and to the doubled dose.
- Diarrhoea presence to the lots which received three and five times he therapeutic dose;
- Limited appetite for fodder and water for the lots who received x3 and x5 times the therapeutic dose;
- Haemoleucogram does not suffers evident changes comparatively with Control lot, the registered values being between the reference

variation limits (with the exception of leucocitary formula);

- Fluctuations by the control lot and dose uncorrelated, of total protein level, of the albumins, globulins, uric acid, with maintaining between variation limits of the reference values;
- Evident increase comparatively to the control lot of the creatinin concentration consecutively to x3 and x5 greater doses administration but without passing the reference values limits;
- Marked increasing of ASAT level and limited for ALAT, comparatively to control lot in the case of E_4 lot, but light to reference values;
- Macro and microscopic changes for liver samples (hepatomegaly, diffuse hepatic degenerescence, nuclear heterochromatinization, vacuolar degenerescence),
- Microscopic and macroscopic renal changes (in nefrons and renal corpuscles, light splenomegaly, caecal sacs distension and brown-yellowish gaseous content to individuals from the x3 and x5 therapeutic doses lots.

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