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. II 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 consecutiv ely 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 E4 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 E3 and E4.

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


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:
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 (E1):** therapeutic dose = 10 mg x kgbw\(^{-1}\) per day,
- **Experimental lot 2 (E2):** two times the therapeutic dose,
- **Experimental lot 3 (E3):** three times the therapeutic dose,
- **Experimental lot 4 (E4):** 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° alcohol, included in paraffin, sectioned to 5\(\mu\) 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 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

<table>
<thead>
<tr>
<th>Lot</th>
<th>Daily b.w. medium gain (g)</th>
<th>Daily fodder consumption (g)</th>
<th>Daily water consumption (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>17.06</td>
<td>34.5</td>
<td>1.6</td>
</tr>
<tr>
<td>E2</td>
<td>13.98</td>
<td>33.78</td>
<td>67.6</td>
</tr>
<tr>
<td>E3</td>
<td>13.66</td>
<td>33.78</td>
<td>52.51</td>
</tr>
<tr>
<td>E4</td>
<td>13.42</td>
<td>29.58</td>
<td>51.9</td>
</tr>
<tr>
<td>C</td>
<td>17.7</td>
<td>34.5</td>
<td>72.96</td>
</tr>
</tbody>
</table>

Note: Accepted reference values are:
- Daily body weight gain (for Cobb, hybrid Hubbard broilers): 23.0 g, 16.7 g [12],
- Daily fodder consumption: 21.2 g, 24-40 g [5], 32.2 g [13].
- Daily water consumption: 76-100 l/1000 chicken [13].

Table 2. Haemoleucogramme for the broilers treated with doxicycline

<table>
<thead>
<tr>
<th>Specification</th>
<th>Arithmetic mean ± Average mean error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (x 10^12/l)</td>
<td>E1 2.33 ± 0.09 E2 2.28 ± 0.063 E3 2.24 ± 0.06 E4 2.06 ± 0.076 C 2.52 ± 0.082</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>E1 31.75 ± 0.77 E2 30.8 ± 1.23 E3 29.9 ± 1.10 E4 28.75 ± 0.92 C 32.3 ± 1.42</td>
</tr>
<tr>
<td>Haemoglobin (g/100 ml)</td>
<td>E1 10.75 ± 0.33 E2 10.4 ± 0.24 E3 10.7 ± 0.27 E4 10.9 ± 0.36 C 10.15 ± 0.17</td>
</tr>
<tr>
<td>Leucocytes (x 10^9/l)</td>
<td>E1 19.03 ± 1.6 E2 18.4 ± 1.71 E3 18.58 ± 1.43 E4 19.68 ± 1.22 C 18.69 ± 0.92</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>E1 92.10 ± 2.42 E2 93.05 ± 3.13 E3 92.85 ± 1.10 E4 92.2 ± 2.84 C 91.5 ± 1.92</td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>E1 4.35 ± 0.51 E2 3.45 ± 0.36 E3 3.7 ± 0.45 E4 4.35 ± 0.43 C 4.6 ± 0.66</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>E1 3.55 ± 0.62 E2 3.5 ± 0.73 E3 3.5 ± 0.48 E4 3.55 ± 0.53 C 3.55 ± 0.7</td>
</tr>
</tbody>
</table>

Note: accepted reference values (x ± DS) for broilers are [14]:
- erythrocytes 2.35 ±0.25;
- haematoctit 26 ±4;
- haemoglobin 7.3 ±1.3;
- leucocytes 26 ±4;
- lymphocytes 63 ±10;
- granulocytes 35.5 ±7.8;
- monocytes 6.1.

The administration of doxicycline (% x lots E1-4/ x lot C) determined low amplitude fluctuations for the level of:
- total proteins (+4.16%-E1; -3.27%-E2; -15.17%-E3; -0.29%-E4),
- albumins (-4.34%-E1; -13%-E2; +8.69% -E3; +13.04%-E4),
- globulins (+15.9%-E1; +2.27%-E2; -13.18%-E3; -6.81%-E4), uncorrelated with dosage and in the reference values limits;
- rise of creatinin concentrations after x3 and especially x5 times therapeutic dose (+28.57%-E3; +42.85%-E4);
- decrease of uric acid level (-63.12%-E1; -34.37%-E2; -50%-E3; -25.62%-E4); uncorrelated with dosage and in the reference values limits;
- progressive growth in parallel with dose increasing of GOT (+6.28%- E1; +6.55%-E2; +9.28%-E3; +14.2%-E4) and pronounced to the five times therapeutic doses,
- ASAT levels increase (+12.12%-E1; +27.27%-E2; +36.36%-E3; +118.18%-E4);
- ALP levels decrease in lots E1:-27.02%; E2:-31.96% and E3:-12.23% and slight increase to lot E4:+1.19%.

The pronounced increase of ASAT levels and limited for ALAT in lot E4, 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 E2, E3 and E4 lots and consisted in:

- light hepatomegaly
- growth in volume of the caecal sacs
- low intensity and diffuse zones of hepatic degenerescence
  - light splenomegaly
  - intestinal vascular ectasia
  - caecal sacs distension and gaseous content
- 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 E1, and E2 lots, there were
not observed tissue structural modifications, but for the E₃ and E₄ lots samples it was observed the hepatocyte’s nuclear heterochromatinization. This can be considered as an outcome of hepatocyte’s decreased metabolic synthesis and is considered as a defensive stage of the hepatic cells against stress aggression. In E₃ and E₄ 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 E₁ and E₂ not emphasised any significant citohistological changes, nephrocytes having a normal aspect with no citoplasmatic vacuoles. Vascular glomerule and Bowman capsule were maintained them integrity. In the case of E₃ and E₄ lots the registered alterations were found in nephrons 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 E₂, E₃, E₄ 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 E₄ lot samples was observed the red pulps’ extension with evident vascular ectasia (see figure 3).

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

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Histologic section through hepatic tissue from E₄ lot’s samples}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure2.png}
\caption{Histologic section through kidney tissue samples from the E₄ lot’s samples}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure3.png}
\caption{Histologic section through spleen in E₄ lot samples}
\end{figure}

4. Conclusions

The doxicycline safety study revealed the following aspects:
\begin{itemize}
\item Good local and general tolerance to the therapeutic dose (10 mg x kgb.w.⁻¹) and to the doubled dose.
\item Diarrhoea presence to the lots which received three and five times he therapeutic dose;
\item Limited appetite for fodder and water for the lots who received x₃ and x₅ times the therapeutic dose;
\item Haemoleucogram does not suffers evident changes comparatively with Control lot, the registered values being between the reference
\end{itemize}
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 corpusules, light splenomegaly, caecal sacs distension and brown-yellowish gaseous content to individuals from the x3 and x5 therapeutic doses lots.

References


10. Antimicrobial Sensitivity Data for the Major Poultry Bacterial Pathogens www.octagon-services.co.uk/articles/poultry