KINETICS OF THE RESIDUE LEVELS OF GATIFLOXACIN IN POULTRY MEAT AND GIBLETS DURING STORAGE

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Abstract
Fluoroquinolones are antimicrobial agents widely used in poultry industry and leads to residues in meat and organs. Gatifloxacin is a fourth generation fluoroquinolone not used in food-producing animals. The aim of the study was to investigate the residues of this antibiotic in poultry meat and giblets during their storage at minus 18°C for 90 days. Chicken (n=18) were treated with gatifloxacin orally at a dose of 10 mg/kg BW for 5 days. Birds were divided into 2 groups and humanely killed on 0-day and sixth day after stopping treatment. Each sample was examined on 0 day, 7-th, 14-th, 21-st, 60, 75-th and 90 day during storage.

The results of the studies on day 1 and 6 showed the highest residues levels of gatifloxacin in skin and liver - 716 μg/kg and 131 μg/kg, 690 μg/kg and 267 μg/kg, respectively. During storage at minus 18 °C the residue levels decreased in different rates in different organs and after one week we had statistically significant difference.

Резюме
Флуорохинолоните са антимикробни агенти широко използвани в птицевъдството и това води до наличието на остатъчни количества от тях в месото и вътрешните органи. Гатифлоксацинът е четвърто поколение флуорохинолон, който не се използва при продуктивните животни в страните от ЕС. Целта на нашето проучване беше да изследваме остатъчните количества от посочения антибиотик в пиленко месо и субпродукти по време на съхранение при -18 °C за 90 дни. Пилетата (n=18) бяха третирани с гатифлоксacin перорално в доза 10 mg/kg чрез водата в продължение на 5 дни. Птиците бяха разделени на 2 групи и хуманно умъртвени на 0-ия и 6-ия ден след спиране на третирането им. Всяка проба беше изследвана на 0 –ия, 7-ия, 14-ия, 21-ия, 60-ия, 75-ия и 90-ия ден от съхранението. Резултатите от 0-ия и 6-ия ден показаха най-високи остатъчни количества от гатифлоксacin в кожата и черния дроб, както следва 716 μg/kg и 131 μg/kg, 690 μg/kg и 267 μg/kg.
По време на съхранение при -18 °C, остатъчните количества намаляват в различна степен в различните органи и след една седмица ние имахме статистически значима разлика.
Key words: chicken, gatifloxacin, residues, storage

Introduction
Antibiotics are used by the poultry industry and poultry veterinarians to enhance growth and feed efficiency and reduce disease. Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and eggs. Antibiotic usage has also enhanced the health and well-being of poultry by reducing the incidence of disease (Donoghue, 2003). Quinolones and fluoroquinolones are a relatively new class of synthetic antibiotics with potent bactericidal, broad spectrum activity against many clinically important pathogens (Abraham, 2003). These agents work through the inhibition of DNA gyrase, interfering with the supercoiling of bacterial chromosomal material (Brown, S. A., 1996). At present only few fluoroquinolones are used in veterinary medicine. Enrofloxacin (Baytril) is the first antibacterial of this family to be available to veterinary medicine. They have a large volume of distribution and are active at very low concentrations (Vancutsem et al., 1990).

Gatifloxacin is a fourth generation fluoroquinolone and it is used relatively recently (Patel et al., 2011). It is a broad-spectrum drug effective against gram-negative, gram-positive bacteria, mycoplasmas and those that are resistant against other agents (Saravolatz, L. D. & J. Leggett, 2003). The numbers of pharmacokinetic studies are being undertaken in domestic animals with a view to adopt this drug in veterinary medicine as well. Its spectrum of activity and pharmacokinetic properties favour its use in veterinary practice (Devada et al., 2012). It is established toxic effect of gatifloxacin and this leads to forbid this antibiotic in domestic animals in EU. Unfortunately, in poultry industry it is used by illegal import and needed adequate controls to prevent the presence of residues to the consumer.

In human medicine, gatifloxacin was shown to be effective in the treatment of acute respiratory infections, including community-acquired pneumonia, acute exacerbation of chronic bronchitis, and acute maxillary sinusitis (Fogarty et al., 1999, Ramirez et al., 1999, Sisniega et al., 1999).

There are a few studies about usage of gatifloxacin in poultry. However, the data about kinetics of the residue levels of gatifloxacin in poultry meat and giblets during storage are lacking. Therefore, the present study was planned to investigate the levels of gatifloxacin residues in poultry meat and giblets during storage at minus 18 °C.

Material and methods
The study was conducted on eighteen broiler chickens. The birds were on two months of age. Six chickens were controls and they weren’t treated with gatifloxacin. Twelve chickens were treated orally with gatifloxacin at a dose of 10 mg/kg BW via the drinking water for 5 days. Water was provided ad libitum. Birds were divided into 2 groups consisted of 6 chickens. First group were humanely killed on 0-day (the day after last administration of levofloxacin) and second group - the sixth day after stopping treatment. Breast muscle, liver, gizzard, heart and skin (with fats) were separated from each carcass. Each organ was divided into seven parts, except heart, which formed six individual samples due to low overall weight. The parts were examined according to days of
storage as follows: 1-st, 7-th, 14-th, 21-st, 60-th, 75-th and 90-th. On the study day all sample were taken out from fridge and thawed at room temperature. Then, they were weighed and homogenized with Maximum Recovery Diluent (HIMEDIA, India) in an amount equal to the mass of the sample and were centrifuged for 15 min at 2500 / min⁻¹ (for liver samples 20 min). The supernatant was collected and dropped (100 µl) on a medium with the test microorganism Escherichia coli ATCC 25922. It was inoculated on plain agar (HIMEDIA, India), previously sterilized and cooled to 50 °C, with concentration of cells 0.5 of McFarland standard. Sterile plates (90 mm) were filled with 14 ml E.coli ATCC 25922 infected agar as described by Okerman et al. 2007. After incubation for 24 h at 37° C, the widths of each inhibition zone were measured from the edge of the sample to the edge of the inhibition zone. Results were processed by GraphPad statistical software.

Results
The results from the study of gatifloxacin residues in meat and giblets are presented on table 1.

Table 1. Gatifloxacin residue levels (µg/kg) in the meat and in the giblets (mean ± SD; n=6) on day 0

<table>
<thead>
<tr>
<th>Tissue samples</th>
<th>Day of storage</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucle</td>
<td></td>
<td>212±24</td>
<td>193±25</td>
<td>98±45</td>
<td>67±28</td>
<td>39±7</td>
<td>39±7</td>
<td>5±0,4</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>690±400</td>
<td>214±84</td>
<td>103±21</td>
<td>48±2</td>
<td>42±3</td>
<td>42±3</td>
<td>42±3</td>
</tr>
<tr>
<td>Gizard</td>
<td></td>
<td>159±400</td>
<td>124±20</td>
<td>101±14</td>
<td>78±15</td>
<td>52±6</td>
<td>52±6</td>
<td>3±0,4</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>170±7</td>
<td>162±9</td>
<td>99±26</td>
<td>-</td>
<td>99±26</td>
<td>93±25</td>
<td>4±0,5</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>716±333</td>
<td>130±15</td>
<td>39±7</td>
<td>34±5</td>
<td>37±5</td>
<td>48±6</td>
<td>0</td>
</tr>
</tbody>
</table>

Microbiological method found gatifloxacin residues in chicken meat and tissues. It is estimated that on day 1 the highest residue levels were in skin (716 µg/kg) and liver (690 µg/kg). During storage at minus 18ºC the residue levels decreased in different rates in different organs and after one week we had statistically significant difference. On day 14 we estimated approximately identical concentrations – between 98 and 103 µg/kg, except in the skin (with fat). At the same day (14-th) in muscle, heart and skin showed values below MRL for fluoroquinolones. Between day 21 and 75 there were no significant decreasing in all tissues, and on the day 90 levels in meat, gizzard, heart and skin were very low (on the limit of detection).

The samples from chicken, slaughtered on day 6 (table 2) after stopping treatment showed lower initial levels of antibiotic on day 1 in all tissues except liver (267 µg/kg) and skin (131 µg/kg). The decreasing of residue levels followed the same pattern as the samples taken on day 0 after treatment, but with lower levels of antibiotic. The most in-
An interesting thing was in liver samples – between 21 and 90 day there were almost the same residue levels both in samples from 0 and 6-th day.

Table 2. Gatifloxacin residue levels (µg/kg) in the meat and in the giblets (mean ± SD; n=6) on day 6

<table>
<thead>
<tr>
<th>Tissue samples</th>
<th>Day of storage</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>60</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>Muscle</td>
<td>24±1</td>
<td>20±1</td>
<td>17±2</td>
<td>14±0,7</td>
<td>11±0,5</td>
<td>8±0,5</td>
<td>2±0,2</td>
</tr>
<tr>
<td>Liver</td>
<td>267±102</td>
<td>52±9</td>
<td>52±9</td>
<td>43±6</td>
<td>41±7</td>
<td>38±7</td>
<td>34±7</td>
</tr>
<tr>
<td>Gizard</td>
<td>24±2</td>
<td>21±2</td>
<td>13±1</td>
<td>7±1</td>
<td>5±0,6</td>
<td>5±0,6</td>
<td>4±0,6</td>
</tr>
<tr>
<td>Heart</td>
<td>19±2</td>
<td>17±2</td>
<td>11±0,6</td>
<td>-</td>
<td>4±0,4</td>
<td>4±0,7</td>
<td>3±0,3</td>
</tr>
<tr>
<td>Skin</td>
<td>131±24</td>
<td>28±12</td>
<td>15±3</td>
<td>10±2</td>
<td>8±1</td>
<td>6±6</td>
<td>6±1</td>
</tr>
</tbody>
</table>

Discussion

Microbiological inhibition tests are the first introduced methods for detecting antibiotic residues which are used even now (Mitchell et. al., 1998; Ferrini, et. al., 2006; Cháfer-Pericás et. al., 2010).

Our previous data about residues of amoxicillin, kanamycine and trimethoprim in poultry during storage were similar to data for gatifloxacin. Pavlov et al. (2005) found a decreasing level of tobramycin during the period of storage. Snapshot of levels of this drug showed initial higher levels in the liver, followed by breast and thigh muscles, with no residues in the muscles on the 30th day.

Dinkov et al. (1998) measured kanamycin and trimethoprim residues in poultry meat and internal organs during their storage at -18 degrees. Kanamycin levels decreased by 60% after 15 days of storage and by 90% after 30 days of storage. Trimethoprim residue levels reduced at a slower rate and substantial quantities were found after 90 days of storage. Boison et al. (1992) proved decrease of benzilpenicillin in bovine internal organs up to 50% from initial level up to 99 day, and zero level of antibiotic in meat. Verdon et al. (2000) found high decrease of ampicillin after 8 month at storage.

Some of our results indicated retention of gatifloxacin residues on the same level in two consecutive periods of the study. O’Brien et.al. (1981) reported that in liver and muscle of cattle in some cases a reduction in the diameter of the inhibition zones are not established.

Gatifloxacin residues in meat and other tissues from chickens decrease during storage at minus 18°C with different rate. The results from our study show that if the samples for analysis in relation to the residue level of antimicrobial drugs are taken during storage, or after freezing, we could estimate lower levels, but at moment of slaughter concentration in tissues could be much higher.
References:


