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Residues of ciprofloxacin and marbofloxacin in poultry meat

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SUMMARY

A total of 105 chicken broiler (antibiotic free) with average weight of 1 kg of each were divided into 3 groups (each of 35 broiler). The examined design was carried out as follow; Group (1) was injected (IV) with 5 mg/kg ciprofloxacin daily for 5 successive days; Group (2) was injected (IV) with 2 mg/kg marbofloxacvin daily for 5 successive days and Group (3) was kept as control without injection any type of antibiotics. After the end of the injection at the 5th day, five chicken of each of the 3 groups were slaughtered after day 1st day, 2nd day, 3rd day, 4th day, 5th day, 6th day, 7th day past last injection.

The breast and thigh muscle samples were screened by ELISA for detection of the residues of ciprofloxacin and marbofloxacin (ppb i.e. µg/kg) as well as examined by sensory test for odour and taste compared with control group. The results revealed that the residues of ciprofloxacin at 1st and 7th day in both breast and thigh muscle were 849 ± 1.7 and 885.6 ± 1.81 and 6.0 ± 0.71 and 10.0 ± 1.22, respectively. It was noticed that the marbofloxacin residues were recorded till the 4th day only and not detected after that. The statistical analysis using t-student test revealed that three were significant increase in ciprofloxacin residues in thigh muscle more than breast muscle in all days. The recommendations for slaughter chickens post ciprofloxacin and marbofloxacin were reported and the results were discussed.

INTRODUCTION

The increasing worldwide demand for animal proteins for human consumption, vast and fast development of animal production, especially in poultry field, has become a very popular business. A variety of antimicrobial types are used in livestock production. Their use inevitably leads to the selection of resistance forms of bacteria in the ecosystem of use. This selection will occur with the uses in livestock production including treatment, prophylaxis and growth promotion. Priority

medical problems arising from the use of antimicrobials in livestock production have been summarized (WHO, 1997). Currently, several quinolones are available for animals, poultry and fish in many countries in the world. Available data indicate that they are also used for diseases prevention in some regions. Quinolone production and usage is estimated to be about 50 tones for proprietary product (mainly USA, European Union, Japan, South Korea) and because of their lower prices, about 70 tones for generic quinolones. For instance, data from china estimate annual quinolone consumption in animals in China alone to be in the range of 470 tones (annual consumption in human medicine in China: about 1.350 tones). An increases in antimicrobial resistance in zoonotic bacteria isolated from animals, food and human (e.g. Salmonella and Campylobacter) has already been reported, but the scope of the problems still needs to be identified and the links between quinolone use in the animals and the occurrence in the infectious disease treatment in humans elucidated (WHO, 1998). Fluoroquinolones are extensively used in the treatment of systemic bacterial infections in poultry, including systemic *E. coli* bacillosis, which is a common disease in turkey flocks. Marbofloxacin has been licensed for use in various mammalian species, but not as yet for turkeys, although its kinetic properties distinguish it from other fluoroquinolone (Ancliya *et al.*, 2006). Marbofloxacin is syenthetic fluoroquinolone, developed for veterinary use only. It has a broad spectrum of activity and bacterial concentration – dependant killing is observed against many Gram negative bacteria (Wolfson, 1985 and Schneider *et al.*, 1996). Ciprofloxacin is one of fluoroquinolone effective in treatment of streptococcal endocarditis, Paeudomonas meningitis, Pneumonia, *E. coli*. To ensure delivery of safe food to consumer, withdrawal times for drugs must respected according to the maximum residual limits established regularly agencies. A withdrawal times of 6 days were calculated based on European Union maximum residual limits (100 µg/kg) for ciprofloxacin and 9 days were calculated based on Japan maximum residual limit (10 µg/kg).

For nearly half a century, immunoassays have been the primary source for detection of analytes of interest in biological samples for both life scince research and clinical diagnostics. This began with the quest to measure insulin levels and culminated in the development of the radioimmunoassay (RIA) by Yallow and Berson (1960). The desire to use less hazardous detection methods than radioisotopes led to the development of the enzyme linked immunosorbent assay (ELISA) by Engrall and Perlmann (1971). The advantages of ELISA are its ease of use, flexibility, the ability of any lab to build assays, and low cost as evidenced by nearly

10.000 studies published per year utilizing the technique (DeJJager, 2005).

This work was planned out to evaluate the residue of marbofloxacin and ciprofloxacin in chicken breast and thigh muscle post I/V injection of 2 mg/kg and 5 mg/kg for 5 successive days after 7 days, as well as to study the sensory characteristics of the aforementioned muscles, respectively.

MATERIAL AND METHODS

A total of 105 chicken broiller (antibiotic free) with average weight of one kgm of each were divided into 3 groups (each of 35 broiler), the experimental design was carried out as follows:-

1- Group 1 (Ciprofloxacin group): This group was injected with 5 mg/kg Ciprofloxacin daily for 5 successive days.

2- Group 2 (Marbofloxacin group): This group was injected with 2 mg/kg marbofloxacin daily for 5 successive days.

3- Group 3 (Control group): This group was kept as control without injection of any type of antibiotics.

After the end of injection at the 5th day, 5 chicken of each group were slaughtered daily to screen the antibiotic residues in breast and thigh muscles as well as the boiling and roasting test for sensory evaluation of breast and thigh muscle of the broiler chicken. The breast and thigh muscle were subjected to the following:-

1- ELISA technique (Bioscientific) for screening the residues of ciprofloxacin and marbofloxacin in breast and thigh muscles as follows:

1.1. Extraction of Ciprofloxacin and marbofloxacin residues in breast and thigh muscle:

One gram of the muscle sample was homogenonized and 4 ml of 70% methanol were added, then vortex for 10 minutes at maximum speed. The samples were centrifuged for 5 minutes at 4.000 x g at room temperature (20-25°C). Exactly 0.5 ml of the supernatant was transferred to a tube, then 0.5 ml of 1 X sample extraction buffer was added and mixed well. Fifty microliter of the extract were used for ELISA assay.

1.2. ELISA protocol:

1.2.1. Fifty microliter of ciprofloxacin and marbofloxacin standard were added in duplicate into different microtitre wells.

1.2.2. Fifty microliter of each sample was added in duplicate into different sample wells.

1.2.3. Antibody # 1 (100 µl) was added and mixed by gentle rocking the plate manually for 1 minute.

1.2.4. The plate was incubated for 30 minutes at room temperature (20-25°C).

1.2.5. The plate was washed 3 times with 250 µl of 1 X washed solution, then the plate was dried on paper towels.

1.2.6. 150 µl were added to the plate and incubated for 30 minutes at 20-25°C.

1.2.7. The plate was washed 3 times with 250 µl of washed solution, then dried.

1.2.8. The substrate (100 µl) TMB was added and mixed gently and incubated 15 minutes at (20-25°C), room temperature.

1.2.9. After incubating, 100 µl of stop buffer were added to stop the enzyme reaction.

1.2.10. The plate as soon as possible was read with 450 nm W.L., then the concentration of the ciprofloxacin and marbofloxacin were calculated.

2. Boiling and roasting test:

It was carried out according to Gracey (1985) to evaluate the odour and taste of both breast and thigh muscles of the injected broilers as well as control groups muscle samples.

Statistical analysis:

Data obtained were statically analyzed using t-student test according to SPSS-14 (2006).

RESULTS AND DISCUSSION

The common use of antibacterial in the treatment of various diseases in animals as well as to control poultry diseases or to improve their performance were attracted many investigators to reveal their residues in edible tissues. Many public health problems may arise from antibiotic residues, which may exist as a result of insufficient withdrawal after drug administration. These include allergic reactions and event as well as development of resistant of bacteria (Booth and McDonald, 1988).

The study of tissue residue was done by injection (I/V) 5 mg/kg ciprofloxacin for 5 successive days. The tissue residue in breast and thigh muscles (ppb) were carried out using ELISA technique. The tissue residues in 1st day, 2nd day, 3rd day, 4th day, 5th day, 6th day and 7th day in both breast and thigh muscle were 849 ± 1.7 and 885.6 ± 1.81, 630.2 ± 1.88 and 669.6 ± 1.6, 474 ± 1.7 and 519 ± 1.98, 249.4 ± 1.63 and 278 ± 0.71, 130.4 ± 0.75 and 147.4 ± 0.87, 82.0 ± 1.67 and 87.8 ± 0.58 and 6.0 ± 0.71 and 10.0 ± 1.22, respectively. The residues of ciprofloxacin in the thigh muscle in all days were significantly higher at $P<0.01$ using t-student test (Table, 1 and Fig., 1).

These results agreed with that recorded by Gorla *et al.* (1997); Corniles (2003) and Cornejo *et al.* (2011).

The MRLs established by European Union (EU) and the Joint FAO/WHO expert commission on food additives (JECFA), where the European Commission has established for chicken tissues, the MRLs for sum Enro and Cipro: 100 ppb for muscle, 200 ppb for liver and 300 ppb for kidney (EC Regulation, 1999). On the other hand, Japan had defined in all chicken tissues MRLs of 10 ppb, while zero tolerance is standard in United States (SanMartin *et al.*, 2007). The

FDA has established muscles as target tissues for residues monitoring in chicken and turkey and set the tolerance at 30 ppb (CFR, 2004).

The ciprofloxacin residues (ppb) in breast and thigh muscle at 5th day were rejected by EC Regulations (1999), (100 ppb i.e. 100 µg/kg) for muscle tissues. So, it was recommended top slaughter the ciprofloxacin treated chicken after the 5th day of last injection.

According to the EMEA (2002), the target tissues for Enro and Cipro are muscles as well as liver and kidney in poultry. On the other hand, the FDA has established that, the edible tissues from which residues deplete most slowly could be considered as the target tissue (Ellis, 2004). The residue of quinolones in muscles was lower than liver and kidneys.

The study of marbofloxacin was carried out as in ciprofloxacin with dose 2 mg/kg. The measuring of residue in both breast and thigh muscles using ELISA technique (ppb) revealed that in 1st day, 2nd day, 3rd day and 4th day the residues were 531± 1.22 and 582 ± 0.95, 382 ± 0.95 and 380 ± 1.14, 125 ± 1.14 and 153 ± 0.25 and 12 ± 0.71 and 15 ± 1.22, respectively. The statistical analysis revealed that residue in thigh muscle is significantly higher than breast muscle at $P<0.01$ using t-student test at 1st day, 2nd day and 4th day while in contrary was insignificant increase in breast muscle than thigh muscle (Table, 2 and Fig., 2).

Poultry diet additives are non-detectable part of poultry industry (Choma, 2003). Most of them have important roles in health and curation of chickens but incorrect use of additives specially drugs may have private problems for consumers (Tajick and Shohreh, 2006). In the developed world, the sustainable rearing of food producing animals depends a great deal on Veterinary Medicines-pharmacologically active compounds. This usage fundamental to achieving a desirable level of animal and public health protection (Banovic *et al.*, 2008).

The administration of fluoroquinolne to food-producing animals without an adequate withdrawal time (WDT) may lead to volatile concentrations of residues in foods destined for human consumption. These residues represent a risk to public health, including simulations on the intestinal microflora and hypersensitivity reactions (Febrega et *et al.*, 2008).

The sensory evaluation of breast and thigh muscle (odour and taste) revealed that there was chemical odour and bitter taste in breast and thigh muscle due to ciprofloxacin residue till the third day of injection only and disappeared at the fourth day. On the other hand, the chemical odour and bitter taste due to marbofloxacin residues in both breast and thigh muscles were recorded only in 1st and 2nd day of injection. The residues in the normal odour and taste in ciprofloxacin residues in both breast and thigh muscles at

4th day were 249.4 ± 1.63 and 278 ± 0.71, while for group at the 3rd day of injection they were 125 ± 1.14 and 153 ± 0.25, respectively.

From previously results of residues and sensory evaluation of chicken breast and thigh muscles post I/V injection of 5 mg/kg chicken wt. ciprofloxacin for 5 successive days and 2 mg/kg marbofloxacin, and the recommended level by EC (1999). The 6th day post last ciprofloxacin injection and 4th day post last marbofloxacin injection were the safe day for slaughtering the treated chickens for good sensory odour and taste and the safe MRLs limit for human health.

In October 2000, FD proposed banning the use of fluoroquinolone in poultry claiming that, these cause infections by resistant bacteria (Habib *et al.*, 2006). Steffenak *et al.* (1994) showed that, cook temperature had no effect on the destruction and concentration of quinolones.

Table (1): Chicken breast and thigh muscle residues of ciprofloxacin after IV injection of 5 mg/kg for 5 successive days concentrations (ppb).

days	Breast muscle residues	Thigh muscle residues
1st	849 ± 1.70	885.6 ± 1.81*
2nd	630.2 ± 1.88	669.6 ± 1.60*
3rd	474 ± 1.70	519.8 ± 1.98*
4th	249.4 ± 1.63	278 ± 0.71*
5th	130.4 ± 0.75	147.4 ± 0.87*
6th	82 ± 1.67	87.8 ± 0.58*
7th	6 ± 0.71	10 ± 1.22*

* Significant at $P < 0.01$ using t-student test.

Table (2): Chicken breast and thigh muscle residues of marbofloxacin after IV injection of 2 mg/kg for 5 successive days concentrations (ppb).

days	Breast muscle residues	Thigh muscle residues
1st	531 ± 1.22#	582 ± 0.95*#
2nd	382 ± 0.95#	380 ± 1.14#
3rd	125 ± 1.14#	153 ± 0.95*#
4th	12 ± 0.71#	15 ± 1.22*#

* Significant at $P < 0.01$ using t-student test comparing with Breast muscle residues.
* Significant at $P < 0.01$ using t-student test comparing with corresponding parameter in ciprofloxacin treated group (Table, 1).

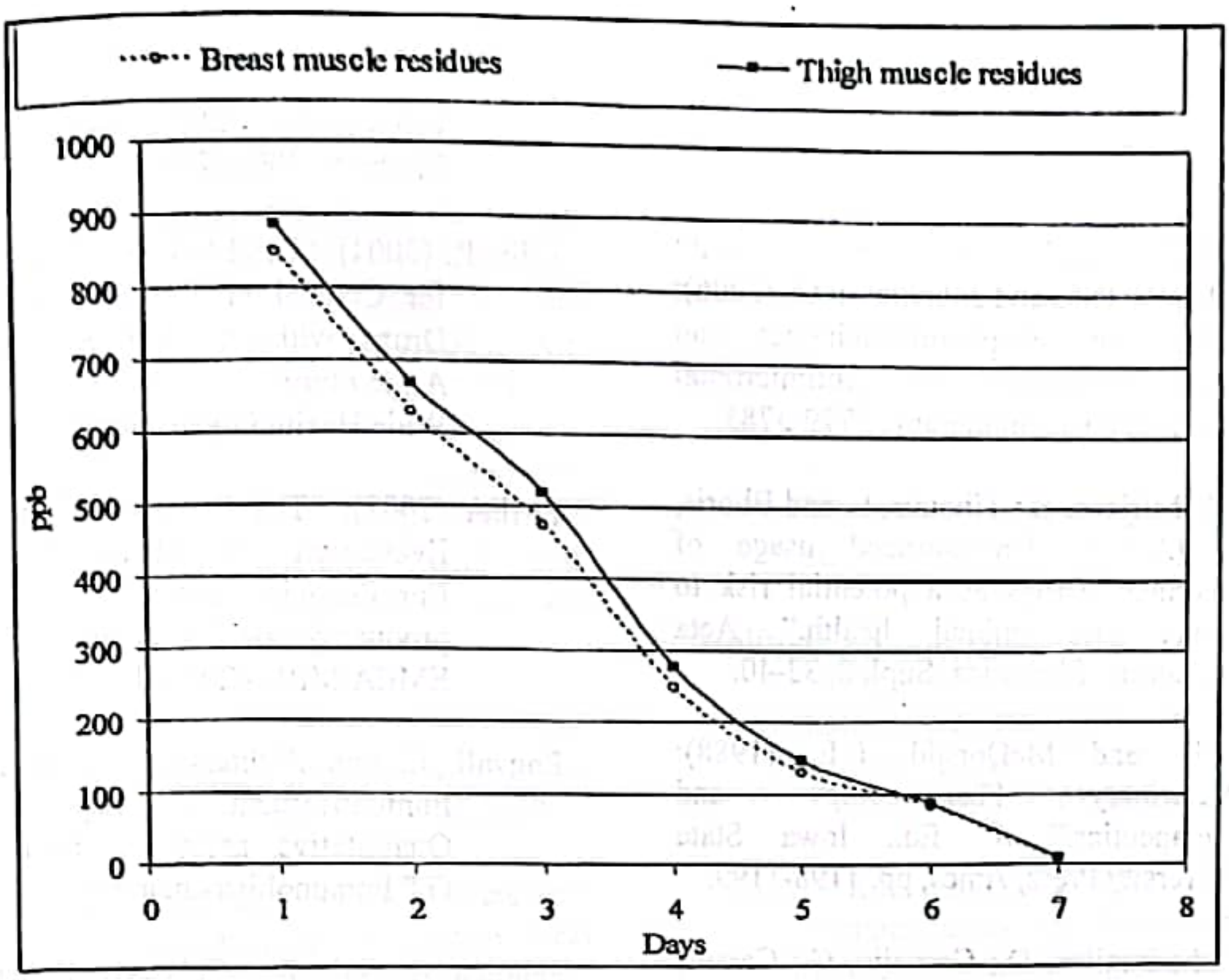

Fig. (1): Chicken breast and thigh muscle residues of ciprofloxacin (ppb).

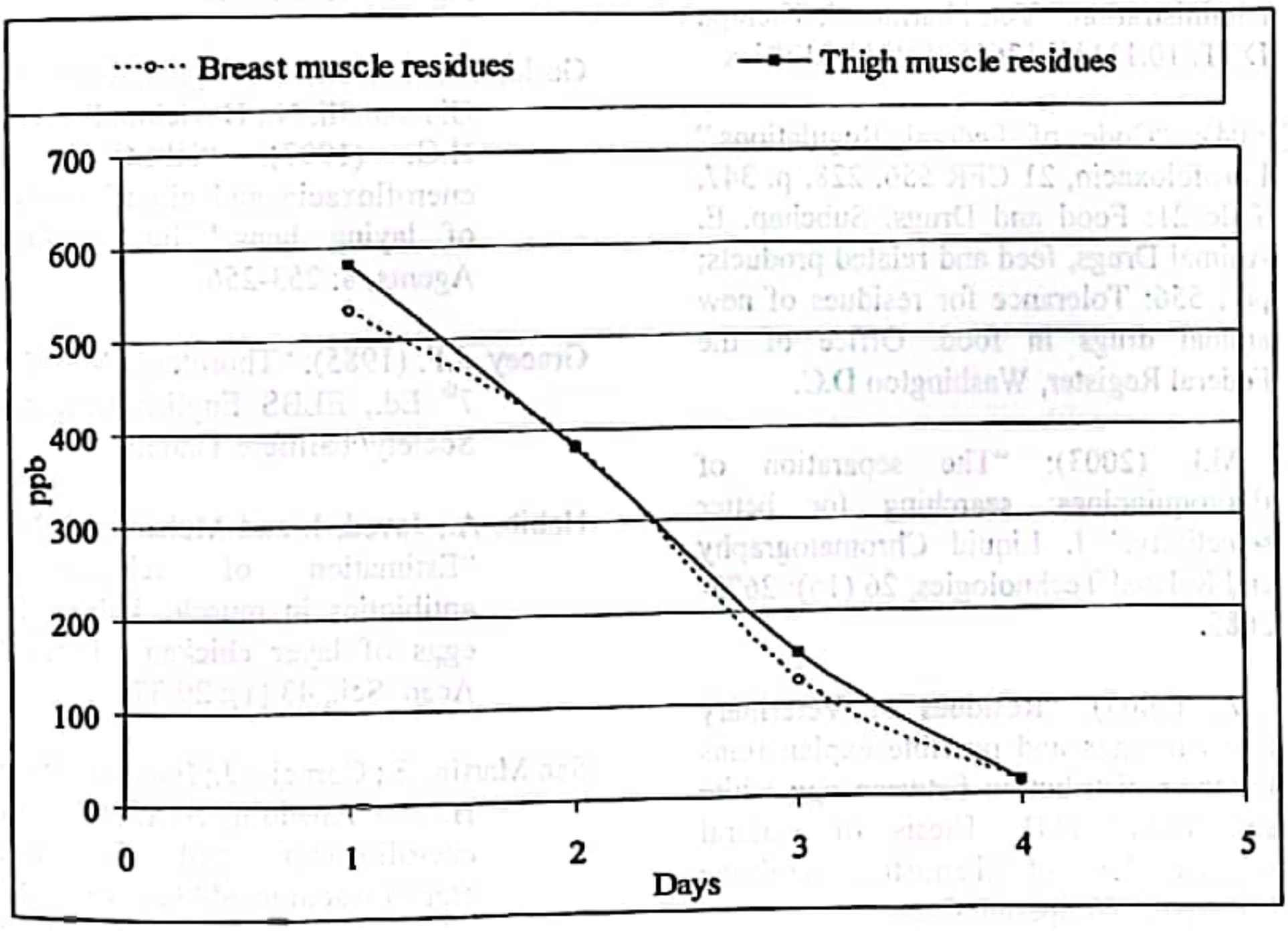

Fig. (2): Chicken breast and thigh muscle residues of marbofloxacin (ppb).

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بقايا السبروفولوكساسين والماربوفلوكساسين فى لحم الدواجن

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تم تقسيم 105 دجاجة وزن 1كجم خالية من أى مضادات حيوية الى ثلاثة مجاميع (كل مجموعة 35 دجاجة) قسمت كما يلى: المجموعة الأولى حقنت عن طريق الوريد بمركب السيبروفلوكساسين بجرعة 5مجم/كجم لمدة خمسة أيام متواصلة، حقنت المجموعة الثانية عن طريق الوريد بمركب الماربوفلوكساسين بجرعة 2 مجم/كجم لمدة خمسة أيام متواصلة أما المجموعة الثالثة تحفظ بدون حقن كمجموعة ضابطة.

ذبحت 5 دجاجات من كل مجموعة من الثلاث مجاميع بعد نهاية فترة الحقن كل يوم حتى اليوم السابع. أخذت عينات من الطيور المذبوحة من عضلات الصدر والفخذ لقياس بقايا السيبروفلوكساسين والماربوفلوكساسين بطريقة الأليزا (ppb i.e. µg/kg) وتم أجراء الإختبارات الحسية للطعم والرائحة لبقايا المضادين فى اللحم مقارنة بالمجموعة الضابطة.

ولقد أوضحت النتائج أن بقايا السيبروفلوكساسين فى اليوم الاول والسابع فى عضلات الصدر والفخذ كانت (849 ± 1.7 ، 885.6 ± 1.81) ، (6 ± 0.71 ، 10 ± 1.22) على التوالى.

وقد لوحظ أن بقايا الماربوفلوكساسين قد سجلت حتى اليوم الرابع فقط ولم توجد بعد اليوم الرابع. ولقد أوضح التحليل الإحصائى بإستخدام أختبار ت أنه يوجد زيادة معنوية فى بقايا السيبروفلوكساسين فى عضلات الفخذ أكثر من عضلات الصدر فى كل الأيام. ولقد أقرت التوصيات بذبح الدواجن بعد الحقن من أى من السيبروفلوكساسين والماربوفلوكساسين ونوقشت النتائج.