The Residue Profile of Ciprofloxacine in Broiler Muscle and Liver

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ABSTRACT: The disadvantages of using antibiotic in broiler are the length of withdrawal time that cause loss production because of prolonged feed consumption and maintenance, and increase the risk of drug residue. The ciprofloxacine is a flouroquinolone antibiotic that recommended in poultry therapy because of its effectiveness to gastrointestinal infection. This research focused on the residue left in broiler muscle and liver resulted from therapeutic application of ciprofloxacine. The experiment conducted to 30 days old of broiler (n = 28) that received the single dose of 50 mg/kg body weight ciprofloxacin intravenously, meanwhile 3 chickens as control did not inject. After drug injection, 3 chickens of each sampling interval were taken to be sacrificed and to collect the abdominal muscle and liver by necropsy procedure. The intervals collecting samples were h1, h8, d 1, d 3, d5 and d7 after injection. The abdominal muscle and liver were minced, homogenized and extracted, then analyzed using high performance liquid chromatography method. The results showed the fluctuated drug levels between intervals, both in muscle and liver. The drug residue level in liver was higher in all intervals compared to muscle. The drug levels in muscle and liver at intervals 1 hour until 7 days post injection were 1.64 ± 0.52 and 7.6 ± 0.60, 3.01 ± 0.06 and 7.15 ± 0.29, 1.31 ± 0.01 and 4.31 ± 0.23, 0.87 ± 0.06 and 7.78 ± 0.35, 1.21 ± 0.07 and 4.01 ± 0.27, 1.18 ± 0.08 and 5.56 ± 0.63 µg/g, respectively. All the levels for both tissues were still above the maximum residue limits according to Indonesian Standard (*Standar Nasional Indonesia*/SNI). It concluded to prolong the withdrawal time of ciprofloxacin application in broiler longer than 7 days, to achieve the safe product to consume.

Keywords: Ciprofloxacin Residue, Broiler, Muscle, Liver

INTRODUCTION

Ciprofloxacine (CIPF) in broiler management was registered under Indonesian agricultural ministry as much 20 commercial products (Anonymous, 2012). As the member of fluoroquinolone group such as enrofloxacin (ENF), the drug was applied intensively in domestic, aquiculture and farm animals for therapeutic purposes. In broiler management, CIPF was formed as metabolite of ENF beside the CIPF therapy applied itself. The drug is effectively against an aerobic negative bacterias, *Brucella, Chlamydophylla, Mycobacterium* and *Mycoplasma* (Maddison *et al*., 2008). The intracellular penetration of drug is similar as high as fluoroquinolone group that make the risk of residue of drug formed in tissues are potentially concerned.

Many researches had deducted to know the ENF residue formed in tissues of poultry and fish in Indonesia, but had not yet documented of CIPF in broiler. The infected *Oreochromis niloticus* with pathogenic bacteria were injected with therapeutic dose of ENF as 10 mg/kg of body weight by oral and muscularly, and after four weeks the tissues still had the excess level of drug of maximum residue levels allowed (Aryanti, 2014). The pharmacokinetic profiles of ENF

in broiler describe the long elimination of half- life in liver and muscles (209.54 and 266.13 h, respectively), which may results the longer withdrawal time of this compound (Ariyani, 2014). The liver and muscle seemed to be the organs that contain the higher ENF levels compare to others, such as blood or kidney. Antibiotic residue in edible animal tissues usually caused by the compound from therapeutic or feed additive agents. Drug residue in edible tissues caused liver, blood and kidney toxicities, allergy reaction, and gut micro flora population imbalance (Haagsma, 1988). The lack of withdrawal time knowing for many compounds also contributes to the residue issue. Many farmers had violated using antibiotic that generate the resistance or residue issue also. The Indonesian maximum limits residue of CIPF in edible tissues has not been established, so the limit was represented by the ENF limit, 0.01 µg/g. This experimentally study was performed to evaluate the CIPF level in broiler muscle and liver in several sampling intervals before it harvest to consumed.

MATERIALS AND METHODS

The 28 New Loghman broilers were maintained since 1- d old chicks, fed with standard feed and had vaccinated, for 30 days and gained at least 1 kg of body weight. The 50 mg/kg dose of CIPF (Tokyo chemical industry(TLI)/Japan) were injected intravenously via brachialis vein for all animals. The three animals were used as control and did not inject. The intervals for tissue collection were h1, h8, d 1, d 3, d 5 and d 7 after injection. For each interval the 3 broilers were taken and sacrificed to find the liver and abdominal muscle. The samples then minced and homogenized, extracted 1 g with 2.5 mL 1% acetonitrile (1 mL anhydrous acetic acid in 100 mL acetonitrile), vortex for 5 minutes then centrifuged 3000 rpm for 5 minutes. Supernatant collected and evaporated with N2 gas. The dry residue then reinstituted with 1.5 mL of phosphate buffer pH 7.4 and 1 mL of hexane, vortex and centrifuged 3000 rpm for 10 minutes. The supernatant were taken, hexane was discharged. The supernatant then reinstituted again for three cycles, and the accumulated supernatant filtered with millipore filter 0.45µm, and kept in freezer until analysis.

The high performance liquid chromatography method had validated as the preliminary study. The results showed good linearity, precision, and accuracy to measure the CIPF in the liver and muscle. Limit of detection (LOD) and quantification (LOQ) were 0.005 and 0.01 µg/g, respectively. The HPLC equipment used was Shimadzu version 6.1 with Shimpack ODS column 5 µm of diameter 150 mm length, pump LC-10 Advp, detector UV SPD-10 A, controller system SCL-10 Avp, oven CTO-10 Avp, and degasser DGU-14. Volume samples injected in loop was 25 µL by microsyringe (SGE, Australia). Mobile phase used in analysis was phosphate buffer solution (0,68 mL of phosphoric acid 85% to 1 L of aquabidest, adjusted pH 3 with triethylamine) and acetronitrile (80:20 (v/v)) (Patriana, 1997). The adjusted flow rate was 1 mL/min, column temperature 30 ˚C and detected at ultra violet wavelength of 278 nm (E-Souza, 2002). The level of drug measured as peak area at certain retention time of chromatogram compare to standard curve of CIPF that validated at preliminary study.

RESULTS AND DISCUSSION

The peak area of CIPF has detected clearly and not overlaps with other peak at 2.8 – 3.0 minute. Drug level measured with standard curve of CIPF and gives the results as mention in Table 1.

Table 1. The residue of CIPF in liver and muscle of broiler after single dose of 50 mg/kg of body weight intravenously

sampling intervals (after drug injection)	concentration (µg/g) (mean±sd)	
	muscle	liver
h1	1.64 ± 0.52	7.60 ± 0.60
h8	3.01 ± 0.06	7.15 ± 0.29
d 1	1.31 ± 0.01	4.31 ± 0.23
d 3	0.87 ± 0.06	7.78 ± 0.35
d 5	1.21 ± 0.07	4.01 ± 0.27
d 7	1.18 ± 0.08	5.56 ± 0.63

h = hour, d = day

All the residue levels shows the higher values in liver compare to muscle. This may reflects the metabolism site of drug majorly occur in liver. As described by Anandon *et al*. (2001), the intravenous application of CIPF gave the fast distribution phase and slowly elimination rate. Based on the volume of distribution and half- life of drug, it showed that CIPF was easily penetrated into tissues and the elimination rate values in broiler were longer than other species. The perfusion rate of liver is better than muscle that leads more drug go to liver following blood flow. Ariyati (2014) stated that after 7 days post injection ENF intravenously with the same dose the residue level in muscle and liver was 0.27± 0.13 and 1.67 ± 0.37 µg/g, respectively. As the metabolite of ENR, CIPF was found in liver and has the same bacterial activity as ENF. The residue of CIPF in liver or muscle (Table 1) were higher than prior research, it may defined that the elimination rate of CIPF was slower than ENF in broiler. At the end of experiment day it has not seen the significantly tendency of decreasing curve although the maximum concentration of CIPF has achieved at 8 h and d3 after injection for muscle and liver, respectively.

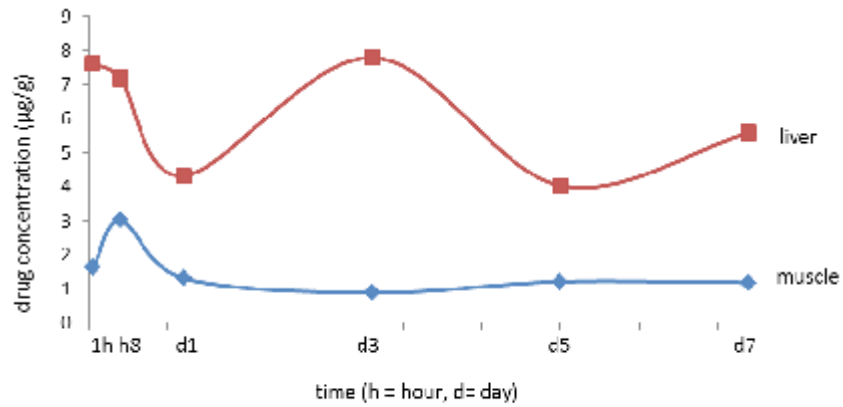

Figure 1. The residue profile of CIPF in liver and muscle after single dose of 50 mg/kg of body weight intravenously

Figure 1 shows the fluctuating concentration of CIPF in the liver and muscle. It seems that elimination phase of drug has not been achieved yet, that may be caused by the high dose given. Otherwise, the dose had been used in the research of ENF residue in broiler by Widiastuti (2008)

and Randall *et al*. (2006), whose were mentioned the dose had good efficacy and antibacterial action. The dose was well tolerated by broiler, that implied with no clinically abnormal or toxicity signs showed during the experiment.

The concerning of using antibiotic in production animals is the residue that may contain in the product. The withdrawal time is the time required after administration of a drug to an animals needed to assure that drug residues in the marketable product is below a determined maximum residue limit (MRL). The pharmacokinetic parameters relatively explain to this term is elimination phase that are half-life or clearance of drug. As it seen at the Figure, the elimination phase (long decreased outline-curve) has no appeared, and the levels of drug in all sampling intervals are still above the MRL (0.01µg/g) for both tissues. It concluded that need longer time to reach the time that assure the drug has no harm effect when it consume. There is no exactly period consideration for withdrawal time, as long the dose and duration of time of therapy was emphasize to eradicate the infection instead of the safe of product. It would take several days until a month to wait the safety product to consume. Chang *et al*.(2009) explained that either high or repeated dose of drug cause the residue in tissues that will remain for a long time, so that it have to extend the withdrawal time. The residue profile of CIPF reflects the high residue level either in muscle and liver in broiler that may need a maximum residue limit for CIPF to be established. Otherwise the application of drug including the dose, duration and administration for therapeutic purpose has to be considering.

CONCLUSIONS

The application of high therapeutic single dose of ciprofloxacin in broiler cause the high level of drug left in muscle and liver. The residue profile showed the fluctuating and high level of drug until seven days post injection. The residue may harm and hazardous, so it needs to be considering the application of drug for therapeutic purposes for yield the safe product to consume. The application of dose of 50 mg/kg body weight in broiler cause the residue as high as 1.18 ± 0.08 and 5.56 ± 0.63 µg/g in muscle and liver at d 7 post injection, respectively. These levels are still above the maximum residue limit for enrofloxacin 0.01 µg/g according to Indonesia National Standard.

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