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Antibiotic Residue Analysis of Duck Meat and Livers in Bogor District

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Abstract: Ducks are one source of animal protein that is widely consumed in Indonesia. The quality and safety of duck meat consumed need to be considered of the hazards of antibiotic residues contained in meat. This study was aimed to determine antibiotic residues contained in the meat and livers. A total of 52 samples of breast meat, thigh meat and livers obtained from 7 groups of farms in 5 districts in the District of Ciomas, Gunung Sindur, Klapanunggal, Jasinga and Jonggol. Samples were taken from each district in proportion to herd of ducks in every district in Bogor. Antibiotic residue test results using bioassay method showed that antibiotic residues occurred in breast meat (7.69%), thigh meat (9.61%) and liver (32.69%). Residue of macrolide found in breast meat, thigh and livers samples. The breast meat samples contained tetracycline residues, while the aminoglycoside contained in the liver samples. Samples that showed positive results in the bioassay method were confirmed by the method of high performance liquid chromatography (HPLC). The breast and thigh meat samples were positive in macrolide residues detected tylosin with concentration of 0.07-0.360 mg/kg. Tetracycline was detected in thigh meat samples with levels of 0.201-0.321 mg/kg.

Key words: Antibiotic Residue · Bioassay · Duck Meat · HPLC

INTRODUCTION

Protein is one of the important nutrients in human survival. Meat is one of the sources of animal protein also contains fat, vitamins and minerals are also needed by the body. Improved economic growth and incomes also increase the demand for animal protein consumption. Ducks *(Anas domesticus)* is a type of protein source, which consumed the meat and eggs. Indonesian's ducks are generally grouped based approach to production of advantages, that laying ducks and broiler ducks. People, who like eating duck meat, are increasing in recent years. This can be seen by the increasing number of restaurants serving menus of duck meat and it caused an increase in the needs of duck meat in the community [1].

According to Directorate General of Livestock and Animal Health Services [2] duck population in 2013 compared to 2012 were increased about 4.41% and increasing of duck meat production about 3.08%. The increase of of duck meat consumption in Indonesia led to an increase in duck meat supply. Duck meat must be produced in good quality and incompliance with the rules of safe, healthy, intact and halal. From the aspect of food safety, besides free of microbial pathogens, good duck meat should be free of residues of antibiotics and other veterinary drugs.

Production and productivity of ducks does not meet the increasing in duck meat demand. This causes farms to increase production and productivity of ducks among others by adding antibiotics, either intentionally or unintentionally on livestock feed or drink. The use of antibiotics in farm is conducted as treatment of disease and also to stimulate the growth of livestock (growth promoters) and feed efficiency, which are generally added in the feed as affixes [3-5]. Improper use of antibiotics can cause high concentration of drug residues in animal body in so that the rest of the metabolites of antibiotics can still be obtained in meat. Antibiotic residues consumed by

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humans would be dangerous, because it cause negative effect to health, including the occurrence of antibiotic resistance to bacteria in human and bacterial resistance in the livestock industry [6, 7].

MATERIALS AND METHODS

Time and Place Research: The study was conducted in June 2013 to January 2014. The study was conducted at duck farms in Bogor, Laboratory of Veterinary Public Health Faculty of Veterinary Medicine Bogor Agricultural University and Quality Control Laboratory and Certification for Animal Product (QCLCAP), Bogor.

Materials: Materials used in this study were obtained of duck meat and livers from farms in Bogor.

Methods

Sampling and Amount of Samples: The method used was a cross sectional survey. In the early stages of research were conducted a survey of duck farms in Bogor includes the name of the farm groups, the number of farmers and farm location. Samples taken are ducks from farm in the district of Bogor. The samples in this study were carried out on the seventh herd in five districts. The sample size was calculated using Win Episcope 2.0 software with of 95% assumption, 50% predicted prevalence, 10% error rate. Sample size thus obtained 52 samples. Sample's amount in each groups were calculated according to the proportional of the total population of duck's breeders and it can be seen in Table 1 [8].

Screening Test Antibiotic Residues: The methods of antibiotic residues screening tests in duck's breast and thigh meat refered to the Indonesian National Standard (SNI) 7424 (2008) about Screening Test Method of Antibiotic Residues in Meat, Eggs and Milk Using Bioassay Method [9]. In this test, residue of antibiotic in the sample would inhibit the growth of microorganisms on agar media. The inhibition could be seen with the formation of barriers area around the paper disc. The diameter of the barriers showed concentrations of antibiotic residues.

In this method, four groups of antibiotics that the penicillins, tetracyclines, aminoglycosides and macrolides were tested. Microbes were used in this test that bacterial spores of *Bacillus stearothermophilus* ATCC 7953 for penicillin group test, bacterial spores of *Bacillus cereus* ATCC 11778 for tetracycline test, bacterial spores of *Bacillus subtilis* ATCC 6633 for aminoglycoside test and vegetative *Kocuria rizophila (Micrococcus luteus)* ATCC 9341 for macrolide test.

Antibiotic Residue Testing Methods by HPLC: The positive results of screening tests on samples of duck breast and thigh meat followed by confirmatory tests using HPLC method. Confirmation test for tetracycline residues refers to Indonesia National Standard (SNI) number 7541:2009 on Testing Methods by High Performance Liquid Chromatography (HPLC) - Part 2: Residues Tetracycline Group in Meat, Eggs, Milk and Its Products [10]. Confirmation test antibiotics residue of aminoglycoside and penicillin group used methods adapted from Agilent Technologies Inc., US [11, 12], whereas for confirmatory testing macrolide antibiotic residue using methods developed by Martos *et al*. [13] and has been modified by QCLCAP.

Data Analysis: The variables measured were the presence of antibiotic residues in breast meat, thighs and livers and the concentration of antibiotic residues in meat duck breast and thigh. The data analysis is to present a descriptive analysis of the test results in tables and figures [14]. Data analysis is performed using Microsoft Excel 2007.

Table 1: Amount of sample each duck farms in Bogor

No.	Sub-district	Village	Number of farms	Sample size breeder
1.	Cariu	Mekar Wangi	15	8
		Karya Mekar	15	8
2.	Gunung Sindur	Pabuaran	10	5
3.	Klapanunggal	Cikahuripan	11	7
4.	Parung Panjang	Parung Panjang	20	10
		Kabasiran	12	6
5.	Ciomas	Laladon	15	8
	Total		98	52

RESULTS AND DISCUSSION

In general, the test results with the antibiotic residue screening tests on bioassay each meat and liver samples were taken from the farm ducks duck in Bogor showed antibiotic residues in breast meat (7.79%), thigh meat (9.61%) and liver (30.77%), which is derived from aminoglycosides, macrolides and tetracyclines. Macrolide antibiotic residues found in samples of breast meat (7.69%), thigh meat (7.69%) and liver (28.85%), while tetracycline antibiotic residues found in thigh meat samples (3.85%) and aminoglycoside residues found in liver samples (3.85%). The test results of antibiotic residues in meat ducks in Bogor can be seen in Table 2 and 3.

According to Bahri *et al*. [15], residues of penicillin group of antibiotics, macrolides, aminoglycosides and tetracyclines were found in meat and chicken liver products. This residue can be derived from the treatment or also feed mixed with antibiotics. Distribution of antibiotics that are less well supervised will result the uncontrolled use in duck farming sector. This situation was also found in the result of this study that showed the macrolide class of antibiotics residues were found at most in the samples of breast meat, thigh meat and livers. The antibiotic residues were found in samples of breast meat, thigh meat and duck liver could be caused due to the use of antibiotics that were not incompliance with the rules and the proper dosage. According to Bedada and Zewde [16] the use of antibiotics were not incompliance with rules, doses, withdrawal time and also the selection of proper antibiotics allows for residues found in animal products.

Positive bioassay test results were confirmed using HPLC. Results of HPLC examination showed tetracycline residues in duck thigh meat samples between 0.201-0.321 mg/kg. The confirmation test results showed tylosin residues in duck breast and thigh meat samples. Tetracycline residues were detected in samples of duck thigh meat. Tylosin's concentration in duck breast meat was between 0.135-0.360 mg/kg. Residue concentrations of tylosin in duck thigh meat were between 0.07-0:08 mg/kg. The antibiotic residues confirmatory test results in samples of breast and thigh meat ducks in Bogor can be seen in Tables 4 and 5. Figures 1 and 2 are chromatograms of antibiotic residues in duck meat samples confirmatory test.

Table 2: Residues of antibiotics in duck's samples from Bogor's duck farming by bioassay method

			Positive Antibiotic Residue by Group (%)			
Type of Samples	Number of samples (N)	Positive Antibiotic Residue (n)	Beta-lactam	Macrolide	Aminoglycoside	Tetracycline
Breast Meat	52	4 (7.69%)	0	7.69	0	0
Thigh Meat	52	5 (9.61%)	0	7.69	0	3.85
Liver	52	17 (32.69%)	0	28.85	3.85	0

Table 3: Antibiotic residues in meat and liver samples of duck by district

			Positive Antibiotic Residue by Group (%)			
Location	Type of Samples	Number of samples (N)	Beta-lactam	Macrolide	Aminoglycoside	Tetracycline
Ciomas	Breast Meat	8	0	0	0	0
	Thigh meat	8	0	0	0	0
	Liver	8	0	5 (62.5)	0	0
Gunung Sindur	Breast meat	5	0	5 (80)	0	0
	Thigh meat	5	0	2 (40)	0	1 (20)
	Liver	5	0	2 (40)	0	0
Klapa-nunggal	Breast meat	6	0	0	0	0
	Thigh meat	6	0	0	0	1 (16.67)
	Liver	6	0	0	0	0
Jasinga	Breast meat	16	0	0	0	0
	Thigh meat	16	0	1 (6.25)	0	0
	Liver	16	0	6 (37.5)	0	0
Jonggol-Cariu	Breast meat	17	0	0	0	0
	Thigh meat	17	0	1 (5.88)	0	0
	Liver	17	0	2 (11.76)	2 (11.76)	0

Table 4: Results of residue testing tetracycline class confirmation by HPLC

			Tetracyclines			
Location	Type of Samples	Number of Samples	Oxytetracycline (mg/kg)	Doxytetracycline (mg/kg)	Tetra cycline (mg/kg)	Chlor tetracy cline (mg/kg)
		MRL	0.1	0.1	0.1	0.1
Gunung Sindur	Thigh meat	1	ND	ND	0.201	ND
Klapa-nunggal	Thigh meat	1	ND	ND	0.321	ND

MRL = Maximum Residue Limit

ND = Not Detected

Table 5: Results of testing confirm the residue class of macrolides with HPLC method

		Macrolides			
Location	Type of Samples	Number of Samples	Tylosin (mg/kg)	Spiramycin (mg/kg)	Erythromycin (mg/kg)
		MRL	0.1	0.05	0.1
Gunung Sindur	Breast meat	4	0.135 – 0.360	ND	ND
	Thigh meat	2	ND	ND	ND
Jasinga	Thigh meat	1	0.07	ND	ND
Jonggol-Cariu	Thigh meat	1	0.08	ND	ND

MRL = Maximum Residue Limit

ND = Not Detected

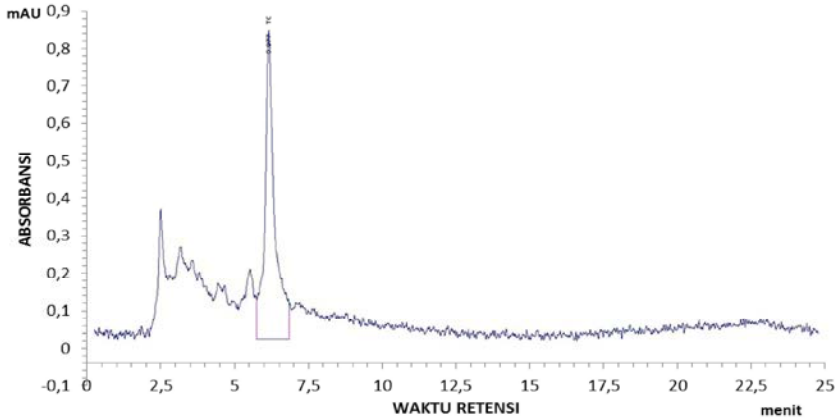

Fig. 1: HPLC chromatogram of tetracycline conformation test

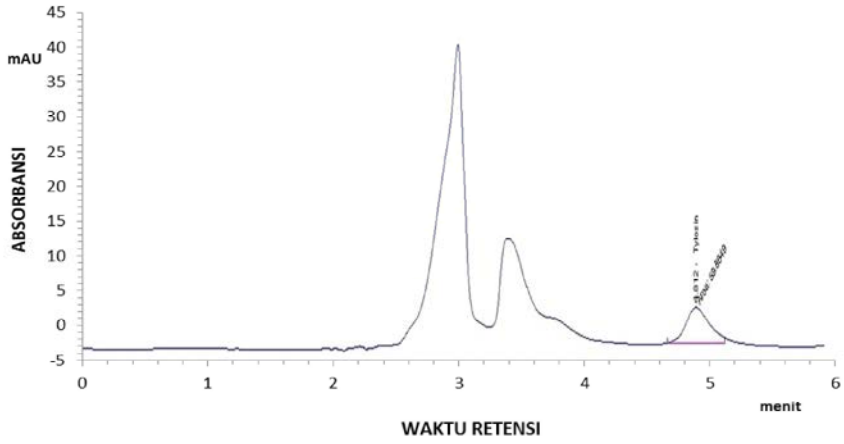

Fig. 2: HPLC chromatograms of macrolide conformation test

Tetracycline types which widely used in poultry farm are tetracycline, chlortetracycline, oxytetracycline and doxycycline. Tetracycline is bacteriostatic antibiotic and works by inhibiting the synthesis of microbial protein. Tetracycline has a broad spectrum, which has the ability to fight a number of pathogenic bacteria. Tetracyclines are widely used, including in Indonesia because it's cheap and easy to obtain [17, 18]. Tetracycline residues in duck thigh meat samples confirmed by HPLC, showed results between 0.201 - 0.321 mg/kg. Concentrations of tetracycline residues were tested above the maximum residue limit (MRL) which 0.1 mg/kg [19]. Widiastuti *et al*. [20] in her study from the analysis of 30 samples of chicken meat collected from Depok, Jakarta and Bekasi, said that 46.67% samples positive containing oxytetracycline with concentration 0.00695-0.04155 mg/kg; 6.67% of positive samples containing tetracycline at a concentration of 0.01328 - 0.05801 mg/kg and 96.67% chlortetracycline positive samples with a range of 0.01472 - 0.04676 mg/kg. The concentration of tetracycline residues in duck thigh samples was higher than the study of Widiastuti *et al*. [20]

Beside tetracycline, macrolide is also widely used in poultry farms. Macrolide is widely used for respiratory diseases treatment also as a growth enhancer in addition of feed mixture. Spiramycin, tylosin and tilmicosin are kind of macrolide that commonly used in livestock and animal health. Tylosin commonly added in the feed as feed additives and also for treatment [21- 23]. Tylosin residues in breast meat samples by HPLC confirmation test showed concentration between 0.135-0.360 mg/kg. Tylosin in samples of duck breast showed that residues above the tylosin's MRL 0.1 mg/kg [19]. Tylosin residue in thigh samples between 0.07-0.08 mg/kg. Tylosin's concentration in the duck thigh samples were below the MRL. According Yuningsih *et al*. [24] that 44.44% chicken meat samples origin from Sukabumi contains tylosin which concentration between 0.0011-0.0845 mg/kg. Tylosin was contained in 66.66% samples from Tangerang which concentration between 0.0006-0.0042 mg/kg. Fifty percent samples originally from traditional market in Bogor was contained tylosin which concentration between 0.0012-0.0094 mg/kg. High levels of tylosin the duck breast samples allegedly because tylosin usage as poultry growth promoters.

Confirmation tests of positive samples showed that tylosin and tetracycline were contained in samples. Beside that, there were undetected antibiotic residues samples, it were possible that the samples contained other antibiotics than the type used as a comparison.

Residues of tetracycline showed that the results were above the MRL; this could be due to less attention to several things including proper rules, dosage and withdrawal time of antibiotics. Improper usage will lead to residues in animal products, toxic effects, also caused allergic reactions and antibiotic resistances [25, 26]. According to Akond *et al.* [27] Kabir *et al.* [28] and supervision of veterinary medicines usage in both the feed manufacturers and farmers need to be strict. Furthermore farmers' knowledge in the veterinary drugs needs to enhance continuously especially in usage and environmental sanitation, so that livestock products which produced have high competitiveness.

CONCLUSIONS

Macrolide residues were found in samples of breast meat, thigh and liver taken from duck farms in Bogor. Tetracycline residues were found in samples of breast meat and the aminoglycoside were found in the liver samples. Confirmatory test by HPLC method on breast and thigh meat samples showed the presence of antibiotic residues of tylosin and tetracycline in thigh meat samples. The presences of antibiotic residues in the samples indicated that the use of antibiotics in duck farm was not well controlled which could become a threat to human health because of antibiotic residue.

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