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Correlation Analysis of Environmental Heavy Metal of Cd Exposure in Liver and Meat of Chicken

Amelia Ramadhani Anshar[1*], Hera Maheshwari[2], Huda S. Darusman[2,3]
[1]Postgraduate Student, Department of Anatomy, Physiology and Pharmacology, Bogor Agricultural University, Jl. Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia
[2]Department of Anatomy, Physiology and Pharmacology, Bogor Agricultural University. Jl. Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia
[3]Primate Research Center, Bogor Agricultural University

*Corresponding author: Amelia Ramadhani Anshar (ameliaramadhanianshar@ymail.com)

Abstract

Cadmium (Cd) belongs to heavy metal with high anthropogenic pollutant and non-essential to physiological function. Regular intake of consuming Cd leads to physiological disorders in poultry animal as well as humans as one who consume it. This study aimed to analyze the Cd exposure in environmental samples (poultry feed and water) and it correlated with the degree of contamination in broiler tissues.In this study, 30 samples of feeds and 30 samples of water, 30 livers and 30 muscles have been examined. The sampling technique using Simple Random Sampling procedure. The results showed that the feed was indicated to Cd between 0.009-0.202 mg/kg and in the water between 0.0068-0.0096 mg / l. Heavy metal of Cd in the livers and muscles was then positively contaminated by Cd between 0.438-0.565 mg / kg and the levels was significantly different (p <0.05) among organs. A strong positive correlation was found between Cd and water levels in liver (r = 0.896). In contrast, a weaker positive correlation (r = 0.596) was found in the water and muscle. In conclusion, Cd contamination in feed and water correlated positively with the increasing amount of the Cd level in organs.
Keywords: *Broiler chicken, cadmium, feed, liver, meat, water*

Introduction

Poultry sectors especially broiler in Indonesia according to Directorate General of Animal Husbandry and Animal Health in 2017 showed that broiler population hasprogressed during the last5 years with an increaseof 4.54% from the previous year. In addition, public awareness of the urgency of food safety is also increasing. Recently, there was increasing of the incidence of poisoning cases due to heavy metal contamination in foodstuffs (Athena *et al*., 2008). Heavy metal of Cd is one of the non-essential anthropogenic metals that is toxic to the body at even low concentrations due to its accumulative nature (Nikic *et al.,* 2009; Bridges *et al*., 2005). Consumed Cd would not show health disorders drastically seen at the firstor second days after consumed contaminated food, but the appearance of clinical disorders would appear in the long term (Patrick, 2003). This is due to the Cd nature that tends to accumulate in the individual body and has a fairly long half-life of 7 to 16 years (ATDSR, 2008). Indonesian National Standard (SNI) 7387: 2009 sets the maximum level of

Cd exposure standard in drinking water is 0.003 mg / l, in chicken bran is 0.2 mg / kg and 0.5mg / kg in poultry organs. Limit tolerance of Cd consumption in its use chronically without causing health problems is 0.0002 mg / kgBW/ day.

The accumulation of Cd in chickens in large quantities will show decrease or inhibition of weight gain and bone growth and may decrease egg production, liver damage, heart failure, brain damage and disorders of the reproductive organs (ovaries and testes) (Vargas *et al.*, 2013; Wang *et al.*,2013; Ferramola *et al.*, 2012; Abib *et al.*, 2011). It is also carcinogenic, interfering with the cardiovascular system, damaging nervous system, the endocrine glands, liver and kidney, prostate, and pancreas (Flora, 2009; Liu *et al.*,2009; Bobocea *et al.*,2008; Barbier *et al.*,2005; Sant'ana *et al.*,2005). Most of Cd metal contamination occurs in broilers chicken's body through the feed. Chicken feed consisted of several types of mixtures such as grains, animal flesh and others. Cd may also be derived from supplementation from supplementation of phosphate minerals and calcium of 0.5 mg/kg.

Based on the phenomenon that susceptible chicken feed is contaminated by Cd metal, it is interesting to study the process of environmental pollution related to chicken health status in Sidrap and Pinrang district. Those districts are the biggest are in eastern Indonesia which has the largest population of broiler chicken. Indeed the environment characteristic would be more meaningful in discussing environmental effects on livestock health. Therefore it is deemed necessary to conduct a study to analyze the profile of Cd content in feed and chicken water by correlating the degree of Cd contamination in broiler tissues.

Materials and Methods

The sampling technique using Simple Random Sampling procedure. The study used 30 livers, 30 muscles, 30 feed samples and 30 water samples. The same retrieval process will be applied to each farm that was used as the sampling site.

There were two kind of data that were taken which is environment data (farm characteristic, poultry feeds and drinking water) and biological data (animal performance and the organs). The environmental characteristics of the farm focused on the study of the geographical location, the topography of the farm, the source of feed and flow of water which will be the source of drinking of livestock.

The Farms Condition

Study was conducted at a chicken farm company located in the districts of Sidrap and Pinrang in South Sulawesi Province. Broiler-type strain of the chickens used in this study was Lawhman MBAI 202 Platinum. The farm located in Pinrang district had an area of 862 m^2. The exact location was in Corowali, Paleteang sub-district, Pinrang. The livestock population was about 4000 to 5000 broilers. The farm area is passed troughbyirrigation connected to the river of Saddang.The drinking water is obtained from the wells, which was suspected to be contaminated with Cd.

The other observed farm which is located in Sidrap district has an area of 910 m^2 with by having chicken population of about 6000 - 7000 broilers. The farm was located at Sereang sub-district of Sidrap district.The farm was surrounded by paddy fields. The characteristics of the environment greatly supportedby the quality of the drinking water.

Testing and determining levels of heavy metal Cd is referred to the Indonesian National Standard (SNI) 01-2354.5-2011. Weigh ± 10 g organs (± 100 ml of water) into the erlenmeyer, add 5 ml of HNO3 and 1 ml of HClO4 to stand by one night. The next day heated at 100 ° C for 1 hour 30 minutes, the temperature was increased to 130 ° C for 1 hour, then the temperature was increased again to 150 ° C for 2 hours 30 minutes (until yellow vapor runs out, if there is yellow steam, it still need heating more time). After the yellow vapor runs out, the temperature is increased to 170 °C for 1 hour; then the temperature is increased again to 200 °C for 1 hour (white steam is formed). Destruction is complete with the formation of a white precipitate. Add aquadest until the mark is tarred, then homogenized. Filter the sample using paper whatman number 42 into a 100 ml reaction tube, then sample ready for analysis using Atomic Absorption Spectrophotometry (AAS) AA-7000 Shimadzu with a wavelength of 228.8 nm.

The data were analyzed using ANOVA One Way, T-test and simple linear regression analysis. The results of the analysis are expected to provide an overview of the relationship between contamination occurring in the feed and water to the degree of contamination of heavy metal Cd in chickens to be consumed by the community.

Results and Discussions

Animal Performance

The body weight and day of harvest of broiler chicken are shown in Table 1. From the table above can be seen that the average age of harvesting time of broiler chicken from Sidrap district was about 26 daysage, while in Pinrang district was 24 days age at harvest. The observation of chicken's body weight in Sidrap and Pinrang districts showed almost a similar average of body weight. The highest body weight in Sidrap with the value of 1.28 kg, while the lowest body weight was 1.19 kg. In Pinrang district showed that the highest body weight gain of 1.33 kg, while the lowest body weight reaches by 1.28 kg.

Table 1. Body weight of chicken sampled from broiler chicken farm at Sidrap and Pinrang districts.

Samples	Broiler Performance			
	Sidrap district		Pinrang district	
	Age at harvest (day)	Body weight (kg/broiler)	Age at harvest (day)	Body weight (kg/broiler)
1	26	1.2	24	1.31
2	26	1.22	24	1.28
3	26	1.27	24	1.31
4	26	1.22	24	1.3
5	26	1.2	24	1.31
6	26	1.23	24	1.32
7	26	1.28	24	1.29
8	26	1.22	24	1.29
9	26	1.28	24	1.33
10	26	1.22	24	1.33
11	26	1.21	24	1.32
12	26	1.19	24	1.29
13	26	1.19	24	1.31
14	26	1.2	24	1.3
15	26	1.21	24	1.28
Mean	26	1.22±0.03	24	1.3±0.02

Cadmium Content in Chicken Organs

The samples of muscle and liver indicated that Cd content was detected in each organ with different concentrations. Mean of concentration in chicken's organs both taken from Sidrap and Pinrang districts are presented in Table 2.The observation of Cd exposure in broiler from Pinrang and Sidrap districts showed a significant difference in each organ ($p < 0.05$). The Cd concentration in Pinrang from the lowest concentration to the highest concentration of muscle 0.48 ± 0.029 mg / kg and liver 0.523 ± 0.018 mg / kg, while in Sidrap muscle 0.461 ± 0.021 mg / kg and liver 0.493 ± 0.013 mg / kg.

Table 2. The Cd deposited in broiler chicken's organs

Samples	CdConcentration*) (mg/kg)			
	Sidrap district		Pinrang district	
	Muscle	Liver	Muscle	Liver
1	0.45	0.486	0.488	0.528+**)
2	0.459	0.506+	0.457	0.499
3	0.467	0.507+	0.498	0.532+
4	0.458	0.488	0.478	0.527+
5	0.447	0.482	0.505+	0.539+
6	0.459	0.506+	0.494	0.531+
7	0.515+	0.516+	0.394	0.498
8	0.459	0.489	0.469	0.499
9	0.507+	0.508+	0.477	0.516+
10	0.459	0.5+	0.528+	0.565+
11	0.451	0.487	0.499	0.539+
12	0.438	0.478	0.477	0.522+
13	0.438	0.47	0.476	0.499
14	0.449	0.484	0.478	0.526+
15	0.457	0.488	0.477	0.522+
Mean	0.461±0.021a***)	0.493±0.013b	0.48±0.029b	0.523±0.018c+

*) Cd consentration calculated per wet weight of mg / kg, value obtained from 3 replicates

**)symbol of (+) means Cd residue above normal threshold > 0.5 ppm (SNI 7387: 2009), limit detection = 0.001 mg / kg.

***)the mean values with the same superscript are not significantly different ($p > 0.05$)

Levels of Cd in muscle tissue in Pinrang farm (0.48 mg/kg) and Sidrap farm (0.461 mg/kg) showed were the levels that were below the quality standard (0.5 mg Cd/kg) and for both farms did not show any significant difference ($p > 0.05$). The muscle was taken from the lateral part of the thigh. High enough Cd concentrations results are thought to be due to a large amount of blood flow that leads to the thigh muscle. Evidenced was reported in a study by Mor *et al.*, (2009) that high concentrations of heavy metals found in the thigh muscles due to the high metabolic activity in the thigh (muscle tissue) that allowed the tissue to be exposed more to heavy metals because of the muscle region (Koronekova *et al.*, 2002).

The accumulation of Cd in the liver was high enough both found in Sidrap and Pinrang farms, reaching up by 0.493 mg/kg and 0.523 mg/kg. Thisis supported by the results of research reported by Trampel *et al.* (2003), Fitzgerald (1985); and Blagojevic *et al.* (2012) who reported that the highest heavy metal accumulation was in the bone, followed by kidney, liver tissue and reproductive organs (ovarian tissue). Ecotoxic researchers explained that in the process of Cd metabolism, Cd was distributed by blood to various tissues (Abduljaleel and Othman, 2013; Mehrotra *et al.,* 2008) with the accumulation rate primarily being in the kidneys at the highest concentrations and then in the liver with relatively lower concentrations. Nangkiawa *et al.* (2015) stated that the liver of one estuary accumulates a heavy metal compound as all the digestive results would be absorbed into the liver via the hepatic portal vein circulation and would enter the hepatocytes. In hepatocytes, Cd would induce the synthesis of albumin and metallothionein proteins (Zalups, 2003), which would then bind and isolate Cd, thereby retaining the toxic effects of Cd in the cell. However, when the liver cells where Cd was isolated die, whether through normal turnover or due to Cd damage the Cd-metallothionein complex would be released into the bloodstream and then accumulate in the liver and kidneys. (Orlowski and Piotrowski, 2003; Klaassen *et al.,* 2009).

Cadmium Content in Feed and Water

Cd content both in feed and water taken from Sidrap and Pinrang farms (Table 3) showed no significantly different. Based on the data above, all the feed samples had Cd contamination well below the recommended threshold and were not significantly different in each region (p> 0.05). Cd metal contamination was detected in wells used as a drinking water source of livestock, which indicates that the value exceeding the recommendation of SNI is > 0.003 mg / l. Detection of heavy metal residues in livestock drinking water sources shows a high enough value and differs descriptively in each region (p <0.05). The highest water contamination is owned by Pinrang district with the valueof 0.0091 ± 0.0003 mg / l and the contamination of drinking water of Sidrap district chicken is 0.00071 ± 0.0002 mg/kg.

Tabel 3. The Cd deposited in feed and water of broiler chickens

Samples	Cd in Feed*) (mg/kg)		Cd in Water (mg/l)	
	Sidrap	Pinrang	Sidrap	Pinrang
Mean	0.102±0.044	0.112±0.015	0.0071±0.0002+a**)	0.0091±0.0003+b

*) Cd consentration calculated per wet weight of mg / kg of feed, and mg/l of water, value obtained from 3 replicates
**)Symbol of (+) means Cd residue above normal threshold > 0.2 mg/kg and >0.003 for water (SNI 7387: 2009), limit detection = 0.001 mg / kg.

Most of the chicken feed contains a small amount of Cd. Grains and grain products are usually the main source of Cd. Feed in the form of grains is indicated to be the cause of Cd in the feed. Also, contamination in feeds due to Cd contamination might be resulted from the additional supplementation in the mineral phosphate feed (Gonzales, 2006).

The decrease in environmental quality can be triggered by the presence of heavy metals that contaminate water, soil and air. Cd metal released from a source of contamination will lead to the atmosphere. Cd will descend to the ground due to air or fall due to rain. Cd that has contaminated the soil layers will lead to high concentrations of Cd metal in ground water. The Cd content will increase in the water retained in the pipe duct. The presence of heavy metals in groundwater is harmful to the health of the immediate environment either directly to the life of the organism, nor indirectly affecting human health (Miranda *et al.,* 2005).

Correlation of Cadmium in Feed and Water with the One Deposited in Organs

The correlation between the contamination in feed and chicken drinking water sources was calculated using linear regression analysis to obtain information about the relationship between free variables and bound variables from samples from Sidrap district farm (Table 4) and Pinrang district farm (Table 5).

Based on Table 4, there was an indication of the positive relationship between Cd metal in feed and water with Cd content in the broiler chicken organs. Correlation of feed and organ in Sidrap farm showed that liver has a highest correlation (r) 0,723 with a determination coefficient of R = 52.29% and the lowest showed by muscle organ by 0,723 (52.29%). The correlation coefficient value of Cd content in the drinking water with Cd content in the liver and muscle respectively were 0.921 (R = 84.83%) and 0.867 (R = 75.26%).

Tabel 4. Coefficient of correlationand determination between Cd content in feed and water with Cd content in broilerchicken's organs obtainfrom Sidrap farm

Organs	Feed		Water	
	r	R%	r	R%
Muscle	0.706	49.86	0.867	75.26
Liver	0.723	52.29	0.921	84.83

Description: r is the correlation value of the T-test, whereas R% is the coefficient of determination. The red box showed the highest correlation value.

Tabel 5. Coefficient of correlationand determination between Cd content in feed and water with Cd content in broilerchicken's organs obtainfrom Pinrang farm

Organs	Feed		Water	
	r	R%	r	R%
Muscle	0.624	38.94	0.77	59.36
Liver	0.879	77.32	0.896	80.37

Description: r is the correlation value of the T-test, whereas R% is the coefficient of determination. The red box showed the highest correlation value.

In Pinrang farm, the correlation of Cd metal as shown in Table 5 showed a positive result. The highest correlation was found in the association between Cd content in the drinking water and Cd content in chicken's liver, which had a coefficient value of 0.896 with determination coefficient value of 80.37% and followed by correlation value in the muscle of 0.77 (R = 59.36%).The coefficient correlation values between Cd content in the feed with Cd content in the muscle and liver respectively were 0.624 (R=38.94) and 0.879 (R=77.32).

Cd metal in drinking water has the highest correlation in renal organ with a value of 0.925. This means having a very close relationship between Cd content in drinking water and kidney (with determination coefficient reaching 85.57%). It is described in US EPA (2006) that the content of Cd metal in water was much more readily absorbed by the body than Cd in feed (5% in water and 2% in feed).

Descriptively the liver tissue showed a relatively high correlation value in both areas with values close to one value. Nangkawi *et al.* (2015) stated that the liver was the accumulated estuarine toxic compound of feed metabolic products in the duodenum which then was absorbed into the liver through the hepatic portal vein.

The Simulation Calculation of Cadmium Contamination

Referring to the data of General Directorate of Livestock Services (DirJen PKH, 2016) that the level of consumption of poultry meat from poultry other than meat, visceral organs is also a part of great interest by the people of Indonesia, one of which is chicken liver. Consuming contaminated liver will affect the biotransformation of metals in the body and deposited in a long time, so over time will cause health status disorders.

Simulation of calculations was done based on the content of Cd metal in the liver, where the liver was selected because it was still an organ that much in demand for consumption by the community. In Table 2 the levels of Cd metal in liver tissue from Pinrang district showed 0.523 mg/kg. The consumption data of visceral organs per capita per week of 2016 is 0.002 kg (Dirjen PKH, 2016). If a person consumes chicken liver as much as 0.002 kg per week with Cd content of 0.523 mg/kg, then the amount of Cd metal consumed is 0.001 mg/person / week. Consumption of liver or other contaminated visceral will contribute considerably to the amount of Cdin the human body. This amount will be greater when more innards are consumed in a long-term and keep in mind that the Cd metal exposure that enters the body has a half-life of 7-16 years, so it will be difficult to reduce the deposit. In addition to the liver, keep in mind that the Cd metal entering the human body can also be derived from the consumption of lung kidney organs and chicken meat, water, air and other foods.

Conclusion

The increase of Cd metal contamination in feed and livestock drinking water increased significantly to the concentration of Cd metal deposited in chicken body tissues. Livers and muscles which examined were positively contaminated by Cd metal. The environmental farm observed at both sites showed that the value of Cd contamination in the water had exceeded the maximum limit of 0.0068-0.0095 mg / l water, while the Cd content in the feed was still in normal concentration between 0.009-0.202 mg/kg of feed. A strong positive correlation was found in the relationship between Cd and water levels in liver and water (r = 0.921), whereas a weak positive correlation (r = 0.624) was found in the relationship between Cd metal content in feed and chicken muscle.

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