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Assessment of lead and cadmium in the eggs of *Gallus gallus* in Ibadan, Nigeria

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Abstract

A study was carried out on the assessment of lead and cadmium in the eggs of *Gallus gallus* in Agbowo and Oluyole area of Ibadan. The aims and objectives of the study were to assess the level of lead (Pb) and cadmium (Cd), study the relationship between lead and cadmium, study the trend of lead and cadmium levels and estimate the health risk in consuming the eggs. Egg samples were collected randomly and fortnightly between July to September 2013 as rainy season and January to February 2014 as representing dry season. Eggs were acid-digested after weighing out 0.5g using weighing balance. Digested egg parts were then transferred to the Buck Scientific's 210VGP Atomic Absorption Spectrophotometer (AAS) for heavy metal analyses of lead and cadmium. Results show that the mean metal concentration in the eggs were in the order for Pb as: Egg yolk<egg white<egg shell, while Cd was egg yolk<egg shell<egg white in the month of July 2013 in Oluyole, while in Agbowo for the same month was Cd: Egg yolk<egg white<egg shell, while Pb was egg white<egg yolk<egg shell. Similar trends were observable with slight variations for other months and locations, but with the egg shell usually having a higher mean concentration for both metals. The mean seasonal pattern of variation in the heavy metals in the egg parts showed that lead was higher for both seasons than cadmium throughout the study. The rainy season values for cadmium and lead were lower than that of the dry season in both Agbowo and Oluyole areas of Ibadan. In Oluyole, Cd was higher during the rainy season (0.1156ppm) than during the dry season (0.1022ppm) with a similar pattern repeating itself for Pb in the egg shell from Oluyole area. The target hazard quotient (THQ) used for assessing health risk exceeded 1 for both lead and cadmium in the eggs thereby indicating a potential health risk associated with the metals. There was correlation between the occurrence of lead and cadmium in the eggs for all the months and locations thereby indicating a relationship between the two metals. The study shows that eggs supplied in parts of Ibadan are impacted by heavy metals such as lead and cadmium with potential health consequences that needs to further investigated.

1. Introduction

Eggs are nutritious and economic foods in the human diet and are included in several food products due to their various functions in the body (Sharkawy and Ahmed, 2002; Sparks, 2006; Thalacker-Mercer *et al*, 2007; Norimah *et al*, 2008;Layman, 2009; Leggli *et al*., 2010, Ruxton, 2010). Global environmental pollution with toxic trace metals has lead to an increase in the investigations concerning contamination of food-stuffs including eggs which represent an important part of the balanced diet

(International Dairy Federation, 1991). Eggs store significant amounts of protein that are useful to the body. The United States Department of Agriculture (USDA) categorizes eggs as meats within its Food Guide Pyramid due to their high protein content (USDA, 2006). Despite the nutritional content of eggs, there are some potential health risks associated with its consumption. These include exposure to environmental contaminants and in some instances individual allergies. Chickens and other egg-laying animals are widely kept throughout the world and mass production of chicken eggs is a global food industry.

Egg proteins make a valuable contribution to the synthesis and maintenance of muscle and indirectly to the regulation of blood glucose levels. Eggs have been used as the standard of comparison for measuring protein quality because of their essential amino acid (EAA) profile and high digestibility (WHO *et al*, 2002; Volpi *et al*, 2003). It provides a nutrient-based source of energy from protein and fat as well as several B vitamins, including thiamin, Riboflavin, folate, B12, and B6, which are required for the production of energy. The unique complementary relationship between the EAA leucine and glucose utilization by muscle implies that a diet rich in the amino acid leucine would be advantageous to those undergoing physical exercise.

Toxic heavy metals affect the quality of eggs that are produced for consumption. It is well known that some heavy metals play different roles in living organisms while others have no known physiological role. There are two main classes of heavy metals namely, essential and non essential metals. Essential metals include: Cobalt(Co) ,Copper (Cu), Iron (Fe),Nickel(Ni),Zinc(Zn) while non essential metals are Arsenic (As),Cadmium(Cd), Mercury(Hg), Lead(Pb) and Uranium (U). Deficiency in any of the essential heavy metals usually results into improper biological function. However, essential heavy metals do become toxic when they are present in excess amounts. Non essential metals also have toxic effects, especially when they enter into the body and exceed a tolerable limit. The diet is the major source of exposure to most of these toxic heavy metals into the body (Pappas *et al*, 2008; Abduljaleel *et al*, 2011). Heavy metals such as Ni ,Co, Cu, Fe and Zn are necessary to balance and maintain proper metabolic activity in living organisms while some other metals like Pb and Cd have no known beneficial physiological function (Ayar *et al*, 2009; Qin *et al* 2009, Rehman *et al*, 2012).

Cadmium gets even more concentrated as it moves through the food chain to reach the higher consumers where it increases in concentration by a factor of approximately 50 to 60 times (White and Finley, 1978; Daniel and Edward, 1995). This is known as biomagnification. Toxic effects of cadmium include hypertension, kidney dysfunction, hepatic injury and lung damage. Cd and Pb are bioaccumulative metals. Therefore, animals with longer life spans have the potential for high concentrations of Cd and Pb in their tissues and organs. As a result of this bioaccumulation and biomagnification, the consumption of meat from older animals could represent an increased risk for ingestion of Cd and Pb (Hogson and Levi, 1997; John, 2002; Fakayode and Olu-Owolabi, 2003). Humans are exposed to cadmium through inhalation and ingestion, although the main health effects recorded in literature are through dietary exposure and occupational exposure. Since cadmium like all elements does not biodegrade in the environment to less toxic products, this contributes to its bioaccumulation potential in the kidneys and liver of vertebrates (IARC, 1993; Baykov *et al*, 1996; Golub, 2005; ICDA, 2005). Diets high in meat especially liver and kidneys or products from marine animals may result in a particularly high risk of intake of cadmium and lead (UNEP, 2008).

Lead has toxic effects on the circulatory, nervous, gastrointestinal and renal systems of the human body. Cadmium is another toxic metal that comes from different sources of food in the environment (Baykov, 1996).Exposure to Pb occurs primarily through inhalation of dust particles, air contaminated with lead and ingestion of foodstuff, water and dust. Inhalation is an important exposure pathway for people in the vicinity of point sources such as lead contaminated sites, countries where leaded fuel is still used and areas where waste from products containing lead is burnt as well as secondary lead (US-ATSDR, 2007). One of the critical human health impacts of lead is neuro-developmental defects in children. Very low levels of lead exposure to children aged between 0-5 years can lead to developmental effects and subsequent lowering of their Intelligent Quotient (IQ). The activities and experiential behavior of children can elevate the exposure of children to Pb when compared with adults in the same setting. In children, exposure begins in-utero due to lead passing through the placental barrier and therefore exposure of pregnant women is also a key concern to children's health (Hu, 1991; ATSDR, 2005; Bergeson, 2008, Schoeter *et al*, 2008, UNEP, 2008; Qin *et al*, 2009, USGS, 2010; NIOSH, 2013).

Given the facts above, the study on the assessment of lead and cadmium levels in the eggs of *Gallus gallus* in Ibadan becomes necessary. The objectives of this study are to assess the level and trend of lead and cadmium, study the relationship in the trends of bioaccumulation between lead and cadmium and estimate the health risk in consuming eggs of *Gallus gallus* in Ibadan, Nigeria.

2. Materials and Methods

2.1. Study Sites

The study sites for the collection of eggs were located in Agbowo (Ibadan North) and Oluyole areas of Ibadan as shown in the map of Ibadan in figure 1 .On the map, Agbowo is located in Ibadan North. Eggs were purchased randomly from 2 different locations in Agbowo (Ibadan) and Oluyole area. Agbowo area is located opposite the main gate of the University of Ibadan .While Oluyole is mainly populated by elites; Agbowo is inhabited by students and middle to low income workers. For the purpose of the work, about 60 eggs (2 each from Oluyole and Agbowo were purchased randomly at three locations during the period of 5 month study (2Eggs X 3 Locations as subsamples X 2 Study sites X 5 months=60eggs). This means that for each of the two study sites, 6 eggs were collected monthly for 5 months, adding up to a total of 60 eggs. The study spanned from July, August, September, 2013 which covered part of the wet or rainy season in Nigeria, and January to Febuary, 2014 that formed the dry season. The study was aimed at assessing the cadmium and lead concentration in the egg shell, egg yolk and egg white during the rainy and dry season periods in Ibadan, Nigeria.

Figure 1. *Map of Ibadan showing Oluyole and Agbowo (Ibadan North) Areas.*

2.2. Sample Preparation for Analyses

After boiling, egg shells were opened carefully and eggs were separated into egg shell, yolk and white. Egg shell were dried and pulverized before weighing out a subsample of 0.5g of each of the sample for digestion in 2mL nitric acid (70%) overnight and hydrogen peroxide (30%) later added and allowed to simmer in a test tube at room temperature. Similarly, egg yolk and egg white were digested. Distilled water was added to the digestate to reach the pre-marked 10mL mark. This digestion followed the procedure used in Nriagulab at the University of Michigan for biological samples. The digested form was taken to the laboratory for the analysis using Buck Scientific 210VGP Atomic Absorption Spectrophotometer (AAS).

2.3. Health Risk Estimation

According to Norimah *et al* (2008), the daily consumption of chicken egg for one person is equivalent without egg shell average (37.2g/person/ day). Cd and Pb were chose to estimate the Target Hazard Quotient (THQ), due to these metal may advertently enter the food chain and pose health to human (Zhuang *et al*, 2009).The methodology for estimation of target hazard offers an indication of the risk level due to pollutant exposure, this method was available in USEPA region 111 Risk based concentration table (USEPA, 2010) which is described by the following equation

$$\text{Target hazard quotient THQ} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{C}}{\text{RFD} \times \text{WAH} \times \text{TA}} \times 10^{-1}$$

EF is 365 for those that consume egg 7 times a week which is common to people of Oluyole because it is inhabited by elite or once per week which is typical of Agbowo because middle class people and student reside there. ED is exposure duration 70 years to average life time, FDI is food ingested rate which is 37.2g/person. C is the metal concentration in egg white and egg yolk only because it is the part consumed. Risk to human from intake of metal contaminated egg was characterized using hazard quotient HQ is a ratio to determine dose of a pollutant to the dose level. If the ratio is less than 1 like in the egg white and egg shell of the egg of Agbowo, there will not be a noticeable risk. Conversely, an exposed population of concern will experience health risks if the dose is equal to or greater than 1.

2.4. Statistical Analysis

All calculations were performed using SPSS for correlation between metals, mean concentration of heavy metals and their standard deviations. Excel was used to plot the histogram showing seasonal variation in lead and cadmium at the two locations.

3. Results

3.1. Heavy Metals (Cd and Pb) Concentration in the Eggs

Table 1. *Mean heavy metal concentration in the eggs in July 2013 (ppm).*

	Oluyole Area		Agbowo area	
	Cd	Pb	Cd	Pb
Egg shell	0.235	0.795	0.01	0.7115
Egg white	0.245	0.4315	0.006	0.3425
Egg yolk	0.022	0.148	0.004	0.4465

The results of the mean heavy metal concentration in the eggs in July 2013 is shown in table 1. The results indicate that in Oluyole, Cd was highest in the egg white (0.235ppm), while the lowest was in the egg yolk (0.022).Lead was highest in the egg shell (0.795) while the lowest mean concentration was recorded in the yolk. In Agbowo, Cd was lowest in the yolk (0.004), while the highest value was recorded in the egg shell (0.01).

Table 2. *Mean heavy metal concentration in the eggs in August 2013 (ppm).*

	Oluyole Area		Agbowo area	
	Cd	Pb	Cd	Pb
Egg shell	0.017	0.3365	0.022	0.0975
Egg white	0.0045	0.19	0.016	0.202
Egg yolk	0.004	0.2705	0.013	0.1315

Table 2 shows the mean heavy metal concentration in the eggs in August 2013.In Oluyole, Cd was highest in the egg shell (0.017) while the least value was in the egg yolk (0.004).Also, Pb was highest in the egg shell (0.3365) while the lowest was in the egg white (0.19). In Agbowo, Cd was highest in egg shell (0.022), while the lowest was in egg yolk (0.013). Pb was lowest in the egg shell (0.0975) and highest in the egg white (0.202).

Table 3. *Mean heavy metal concentration in the eggs in September 2013 (ppm).*

	Oluyole Area		Agbowo area	
	Cd	Pb	Cd	Pb
Egg shell	0.095	0.261	0.2485	0.149
Egg white	0.1245	0.2035	0.2535	0.252
Egg yolk	0.1625	0.2215	0.2585	0.255

In September 2013 in Oluyole Area, the mean Cd concentration was highest in egg yolk (0.1625) and lowest in the egg shell (0.095), while Pb was highest in the egg shell (0.261) and lowest in the egg white (0.2035). In Agbowo Area, Cd was highest in the egg yolk (0.2585) and lowest in the egg shell (0.2485), while Pb was highest in the egg yolk (0.255) and lowest and lowest in the egg shell (0.149).

Table 4. *Mean heavy metal concentration in the eggs in January 2014 (ppm).*

	Oluyole Area		Agbowo area	
	Cd	Pb	Cd	Pb
Egg shell	0.1305	0.1365	0.955	0.354
Egg white	0.1525	0.19625	0.1165	0.252
Egg yolk	0.594	0.1375	0.2585	0.255

Table 4 shows the mean heavy metal concentration in the eggs in January 2014.In Oluyole, the mean Cd concentration was highest in the egg yolk (0.594) and lowest in the egg shell (0.1305), while Pb was highest in the egg white (0.1965) and lowest in the egg shell (0.1365). In Agbowo, Cd was highest in the egg shell (0.955) and lowest in the egg white (0.1165), while Pb was also highest in the egg shell (0.354) and also lowest in the egg white (0.252).

Table 5. *Mean heavy metal concentration in the eggs in February 2014 (ppm).*

	Oluyole Area		Agbowo area	
	Cd	Pb	Cd	Pb
Egg shell	0.074	7.235	0.0705	13.27
Egg white	0.058	12.105	0.042	6.275
Egg yolk	0.0225	5.105	0.1045	7.865

As shown in table 5, the mean heavy metal concentration in Oluyole shows that Cd was highest in the egg shell (0.074) while the least was in the egg yolk (0.0225).Pb was highest in the egg white with a value of 12.105ppm, while the least was in the egg yolk (5.105).In Agbowo, Cd was highest in the egg yolk (0.1045) with the lowest value in the egg white (0.042), Pb was lowest in the egg white (6.275),while the highest was in the egg shell (13.27).

3.2. Seasonal Variation of the Heavy Metals in the Eggs

The mean Cd and Pb concentration in the eggs at Oluyole and Agbowo areas from July to September 2013 were computed as representing the rainy season, while values from January to February 2014 represented the dry season assessment. By using Excel, the histograms were drawn with the data values shown on the charts for comparison. As shown in the mean seasonal pattern of variation in the heavy metals in the egg parts (egg shell, egg white and yolk) displayed in the figures 2 to7 below, lead was higher for both seasons than cadmium throughout the study. The rainy season values for cadmium and lead were lower than that of the dry season in both Agbowo and Oluyole.

For instance, in figure 1, Cd was higher during the rainy season (0.1156ppm) than during the dry season (0.1022) with a similar pattern repeating itself for Pb in the egg shell from Oluyole area. This trend was observed in all the egg parts (shell, white and yolk) from the two study areas throughout the study as shown in the other figures: 2-6.

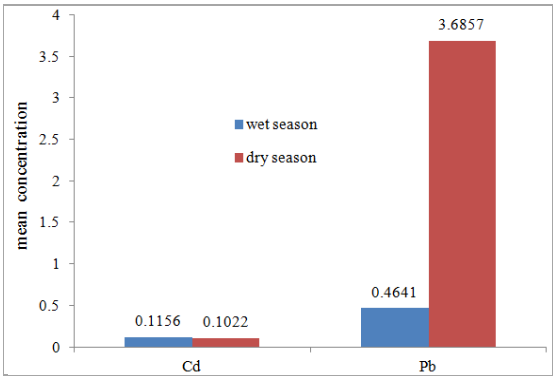

Figure 1. *Seasonal variation of cadmium and lead (ppm) in the egg shell in Oluyole Ibadan (ppm)*

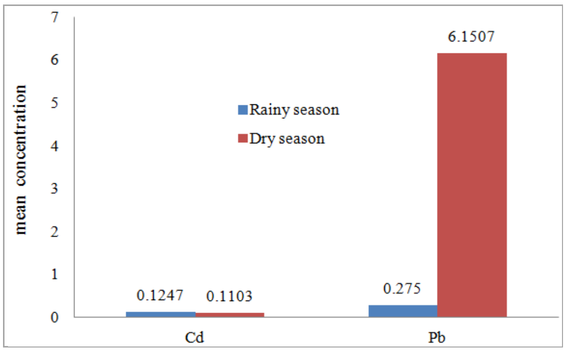

Figure 2. *Seasonal variation in the cadmium and lead (ppm) in the egg white in Oluyole*

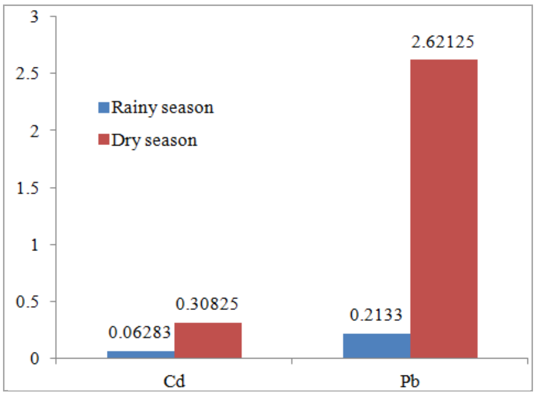

Figure 3. *Seasonal variation of cadmium and lead (ppm) in the egg yolk in Oluyole*

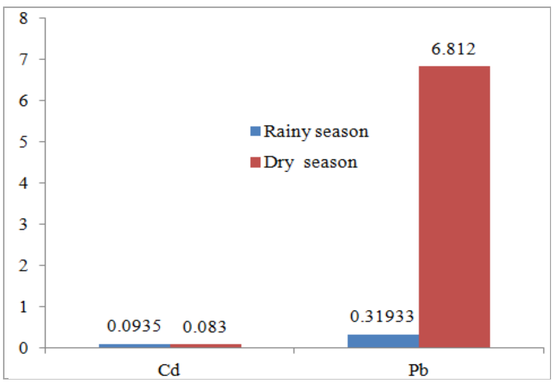

Figure 4. *Seasonal variation in cadmium and lead (ppm) in the egg shell in Agbowo*

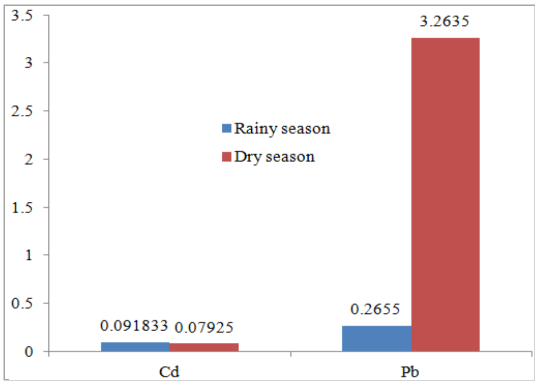

Figure 5. *Seasonal variation of cadmium and lead (ppm) in the egg white in Agbowo*

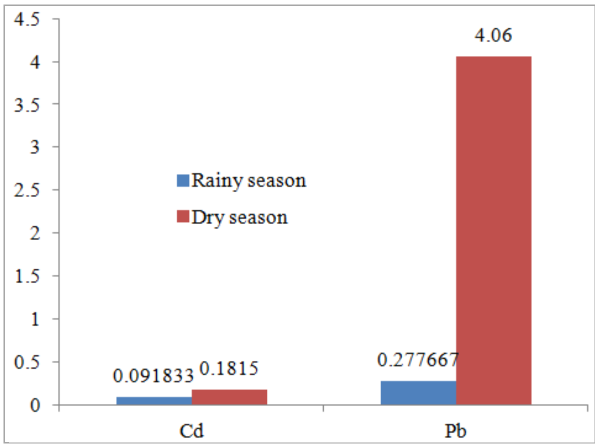

Figure 6. *Seasonal variation of cadmium and lead (ppm) in the egg yolk in Agbowo*

3.3. Pearson's Correlation Coefficient between Cadmium and Lead Variation in the Eggs

Results of the correlation between Cd and Pb in the egg shell, egg white and egg yolk for the study areas of Oluyole in the months of July 2013 ,August 2013,September 2013,January 2014 and February 2014 were with the following negative r values at $P<0.05$:-0.967,-0.213,-0.971,-0.955 and -0.916 respectively .In Agbowo, the r values were 0.922,0.310,0.995,-0.921 and -0.141 for the months of July 2013, August 2013,September 2013, January 2014 and February 2014 respectively. A detailed summary of the correlation coefficients and descriptive statistics is provided in the appendices.

3.4. Health Risk Assessment

The health risk estimation or assessment values for consuming metal contaminated eggs were above 1 for both Pb and Cd. This is the recommended minimum, which if exceeded poses a health threat to humans. In addition the mean concentration of Cd and Pb were compared to World Health Organization guideline limits, SPIRULINA for Japan and United States farmers as well as local standards given by Federal Environmental Protection Agency (FEPA, 1991) and were found to have both exceeded the limits.

4. Discussion

4.1. Distribution of Heavy Metals in the Egg Shell, Egg White and Yolk

The high cadmium and lead concentration in the egg shell can be attributed to its role as an excretory storage organ and the affinity of these metals to calcium which is the major constituent element in the egg (Brady and Holum, 1988, Hogson and Levi, 1997, Nagabhushanam, *et al*, 1999).Of great concern to public health is the mean metal concentration in the egg white and yolk that are consumed by adults and used for weaning babies.

4.2. Seasonal Variation of Heavy Metals in the Eggs

The high seasonal lead levels in the egg parts in the study can be attributed to the diversity of sources of the metal when compared with cadmium. It is also attributable to metallic ion mobility in the environment. Leaded fuels are still in use in the country and accounts for the thousands of tons of lead outfall into the environment. Similar variation patterns in heavy metals had been reported by Mora (2003); McRoy *et al* (2005); Swaileh and Sansur, 2006; Waegeneers *et al*, (2008); Van Overmeire *et al* (2009) and Abduljaleel *et al*, (2011).

Dilution factor could be accountable for the low rainy season mean values of the cadmium and lead in the eggs especially from aquatic sources through drinking water. As a result, the chickens become exposed to a copious and dilute concentration of the heavy metallic ions that is bioavailable to the animals. It can be asserted that metal ions in the water sources become concentrated due to evaporation in the dry season thereby becoming more concentrated in the tissues of the chicken and in eggs (Nagabhushanam *et al*, 1999).

4.3. Health Implication of Consuming Eggs Supplied in Ibadan

Although the World Health Organization (WHO) guideline limitation for lead is 0.05ppm, it can be found that most of the mean concentration exceeded this limit and is therefore a public health threat. This means that studies need to be carried out on the chicken feeds to know whether they are contaminated or not and to assess other sources of exposure (WHO/FAO, 1989, Wageneers *et al*, 2008) .However, the limits for cadmium is not listed in this WHO guideline limitations, although SPIRULINA guideline for Japanese and United States farmers list it as 0.05ppm like the WHO (2008) guideline, while lead is <1ppm. Similarly, the local guidelines of the Federal Ministry of Environment (from defunct FEPA) specify <1ppm (FEPA, 1991).This means there is palpable risk in consuming eggs during the dry season in Agbowo and Oluyole in Ibadan. This health risk could be due to ground water sources used as drinking water for the chickens, inhalation of contaminated fly ash from landfills, the feeds or poor handling of the eggs. For instance more studies need to be carried out to trace the livestock farms supplying the eggs, the feeds they use and how often, water sources for the chicken and air exchange facilities in the pens with the open atmosphere .Potential sources of exposure to heavy metals in the environment needs to be assessed on a periodic especially during the dry season which shows high mean metal concentration in the eggs.

5. Conclusion

In conclusion in this study, Pb and Cd are more concentrated in the egg during the dry season than in rainy season; therefore consumers of egg may be susceptible to risk in this season. Oluyole occupant are at higher risk because it is dominated by elites who can afford and consume eggs 7 times in a week therefore, children and babies are at more risk because the nutritional value of egg is high, the babies need it for their growth unlike Agbowo inhabitant which is dominated by middle class people and student, therefore the chances of heavy metal uptake in eggs will be low due to the comparatively lower income status. The role of government can come into play to reduce high risk of heavy metals by keeping the people informed about the health risk involved in egg consumption, and to check the rate at which egg are consumed especially in the dry season. The government can educate poultry farmers on the various ways of handling chickens in order to regulate heavy metals in the body of *Gallus gallus*.

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