Determination of the Toxic Level of Dietary Mercury and Prediction of Mercury Intake and Tissue Mercury Concentrations in Broiler Chickens Using Feather Mercury Concentrations

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Primary Audience: Nutritionists, Feed Mill Managers, Food Safety Managers, Broiler Producers

SUMMARY

The current experiment was conducted to determine the toxic level of dietary mercury (Hg) and to predict Hg intake and tissue Hg concentrations in broiler chickens from feather Hg concentrations. A total of 800 3-d-old broiler chickens (400 male and 400 female birds) were allotted to one of 80 battery cages in 2 × 5 factorial arrangements of sex and 5 concentrations of Hg in diets with 0, 50, 100, 250, or 500 mg/kg. The mercury chloride was used to increase Hg concentrations in diets. Experiment lasted for 32 d. There were no interactions between sex and dietary treatments for all measurements. A greater than 250 mg/kg Hg had negative effects on broiler performance. The one-slope broken-line analysis with dietary Hg concentrations and BW gain revealed that a greater than 209 mg/kg Hg in diets was toxic to broiler chickens. Feather Hg concentrations were greater than liver and breast Hg concentrations across all treatments, indicating that the feather is the most responsive tissue to dietary Hg concentrations. Accordingly, the equations for predicting daily Hg intake and Hg concentrations in the liver and breast were generated from Hg concentrations in the feather. Resulting equations indicated that feather Hg concentrations are good predictors of both Hg intake and Hg concentrations in the liver and breast. In conclusion, the toxic level of Hg is near to 200 mg/kg in broiler diets. The Hg intake and Hg concentrations in the liver and breast can be precisely predicted from Hg concentrations in the feather.

Key words: broiler chicken, mercury concentration in body tissue, prediction equation, toxicity of dietary mercury

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DESCRIPTION OF PROBLEM

Mercury (**Hg**) is one of the toxic heavy metals and is widely used in industrial production of

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fluorescent lamps, thermometers, and other electronics. It is reported that the amounts of global Hg emissions ranged from 3,600 to 5,300 ton per year [1–3]. Therefore, humans and animals can be easily exposed to Hg-contaminated food, air, soil, and water. European Food Safety Authority (EFSA) reported that Hg contamination in various food and feed materials was prevalent in many countries and the contamination levels of Hg ranged from 0.1 to 50 μg/kg in human foods and from 12 to 53 μg/kg in animal feeds [4, 5]. Recently, the use of Hg in industrial processes is steadily increased, and thus, the reports of Hg poisoning in humans and animals will be mounted, which lead to a great public concern.

The Hg in the environment typically enters the body through the gastrointestinal tract, the respiratory system, and the skin [6]. Significant exposure to Hg results in various health problems including disturbances in the renal and hepatic function and can negatively influence the nervous systems in humans and animals [4]. For this reason, the World Health Organization (WHO) has designated Hg as one of the 10 hazardous chemicals posing the major threat to public health [7].

Several experiments in poultry have investigated the toxic level of Hg in diets [8–10]; however, the results of those previous studies are inconsistent. Further, the toxic level of dietary Hg determined in previous experiments is still questionable to apply for the current poultry industry because of continuous changes in poultry genotypes and rearing conditions. Moreover, a rapid and accurate estimation of tissue Hg concentrations in poultry is critical to identify Hg contamination in poultry products for the food safety. In poultry, the feather can be a good target tissue to access body Hg status because heavy metal concentrations in the feather are highly responsive to changes in environmental exposure of heavy metals. In addition, the feather is easily and continuously collected in an animal-friendly way because of no need for sacrificing birds [11, 12]. However, despite the benefits associated with measuring heavy-metal exposure using feather samples, few experiments have analyzed heavy-metal contaminations in poultry. In addition, no previous experiments have performed to predict Hg intake and Hg concentrations in the edible tissue of poultry from Hg concentrations in the feather.

Therefore, the objectives of the present experiment were to determine the toxic level of dietary Hg and to derive equations for predicting Hg intake and tissue Hg concentrations from feather Hg concentrations in broiler chickens.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the Institutional Animal Care and the Use Committee at Chung-Ang University.

Diets, Animals, and Experimental Design

A total of 1,000 1-d-old Ross 308 broiler chickens were obtained from a local hatchery [13] and were transferred in an environmentally controlled room. Birds were fed the commercial diets for 2 d. After all chicks were weighted at 3 d of age, 200 birds with extremely high and low BW were discarded. The remaining 800 birds (400 male and 400 female birds) were randomly allotted to one of 80 battery cages (10 birds per cage) with a similar average BW (initial BW = 65.2 $\pm$ 5.69 g) in 2 $\times$ 5 factorial arrangements of sex and 5 dietary treatments. A 2-phase feeding program with a starter diet from 3 to 21 d and a grower diet from 21 to 35 d was used. Within each phase, a commercial-type basal diet was formulated to meet or exceed the NRC requirements for broiler chickens (Table 1) [14]. The mercury chloride ($HgCl_2$; $\geq$73.9%) [15] was added to the basal diet at 5 different concentrations of 0, 50, 100, 250, and 500 mg/kg Hg in replace of the celite. The analyzed concentrations of Hg in experimental diets for starter diets were 71, 105, 246, and 481 mg/kg and for grower diets were 94, 131, 234, and 459 mg/kg, respectively (Table 2). All diets were fed to the birds in a mash form on an ad libitum basis for 32 d. The room temperature was maintained at 30°C during the first week and then gradually decreased to 24°C at the conclusion of the experiment. Birds were raised in a 24-h lighting schedule throughout the experiment. The BW gain and feed intake (**FI**) were recorded at the conclusion of the experiment. The FE (g/kg) was calculated by dividing BW gain with FI after adjusting for mortality [16].

Data Collection and Chemical Analysis

At the end of the experiment, 1 bird per replicate with a BW close to the replicate mean BW (i.e., 8 male and 8 female chickens per treatment) was selected and weighed from each cage for sample collection. The selected birds were euthanized by CO_2 inhalation and immediately dissected. The liver, breast, and feather were collected for analyzing Hg concentrations in those tissues. The feather samples were obtained from the belly and back sides of birds. The tissue samples were weighed and digested for analyzing Hg concentrations according to the method described by Rajkowska et al. [17] with minor modifications [18]. Briefly, approximately 1 g of diets, liver, and breast and 0.1 g of feather samples without rachises and calamus were added with 10 mL of HNO_3 and 5 mL of $HClO_4$ in 500 mL Kjeldahl flask, and then digested using a Kjeldahl digestion apparatus until the color became transparent. Each 500-mL Kjeldahl flask was then removed from Kjeldahl digestion apparatus and maintained at room temperature to allow them to cool following digestion. The digested solutions were then filtered by Whatman filter paper (Whatman® Grade 42) [19]. The content of filtrates was used to determine Hg concentrations using an inductively coupled plasma spectrometer [20].

Statistical Analysis

All data were analyzed by ANOVA as a completely randomized design using the PROC MIXED procedure of SAS [21]. The replicate was considered as an experimental unit for all analyses. The initial statistical model included sex, dietary Hg concentrations, and their interaction. However, there were no significant interactions between sex and dietary Hg concentrations for all measurements. In addition, no significant main effects of sex were also observed for all measurements, except that male chickens had greater BW gain and Hg concentrations in the liver than female chickens. Thus, sex and interaction terms were removed from the model and dietary Hg concentrations were only included in the final model. LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant [22]. Preplanned orthogonal polynomial contrast tests were performed to investigate the linear and quadratic effect of increasing Hg concentrations in diets [23]. The IML procedure of SAS was used to determine contrast coefficients for unequal-spaced Hg concentrations in diets.

The one-slope broken-line analysis [24, 25] was performed to predict the toxic level of Hg in broiler diets based on BW gain using the

Table 1. Composition and Nutrient Content of Experimental Diets.

Items	Starter diets (3 to 21 d)	Grower diets (21 to 35 d)
Ingredients (%)		
Corn	49.89	50.05
Soybean meal (45% CP)	29.67	22.35
Wheat	4.50	10.00
Corn gluten meal	6.34	7.00
Tallow	3.80	5.34
Salt	0.30	0.30
Monodicalcium phosphate	2.00	1.74
Limestone	1.49	1.40
Threonine (98.5%)	0.25	0.20
DL-Methionine (98%)	0.45	0.36
Lysine HCl (78%)	0.60	0.55
Choline (50%)	0.10	0.10
Mineral premix[1]	0.10	0.10
Vitamin premix[2]	0.10	0.10
Sodium bicarbonate	0.10	0.10
Celite	0.20	0.20
Antioxidant	0.01	0.01
Coccidiostats	0.10	0.10
Total	100.00	100.00
Nutrient content[3]		
AME_n (kcal/kg)	3,053	3,215
CP (%)	22.39	20.01
Lysine (%)	1.36	1.16
Methionine + Cysteine (%)	1.04	0.91
Calcium (%)	1.00	0.90
Non-phytate phosphorus (%)	0.50	0.46

[1]Provided per kilogram of the complete diet: Zn (as ZnO), 100 mg; Mn (as $MnSO_2 \cdot H_2O$), 120 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 60 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 16 mg; Co (as $CoCO_3$), 1,000 μg; I (as $Ca(IO_3)_2 \cdot H_2O$), 1.25 mg; Se (as Na_2SeO_3), 300 μg.

[2]Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 13,000 IU; vitamin D_3, 5,000 IU; vitamin E (from $_{DL}$-α-tocopheryl acetate), 80 IU; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 6 mg; vitamin B_{12}, 20 μg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 200 μg; niacin, 60 mg.

[3]Calculated values from NRC [14].

Table 2. Expected and Analyzed Mercury (Hg) Concentrations in Experimental Diets.

Treatments	Expected Hg concentrations (mg/kg)	Analyzed Hg concentrations (mg/kg)	
		Starter diets	Grower diets
0	0	1	2
50	50	71	94
100	100	105	131
250	250	246	234
500	500	481	459

nonlinear regression (NLIN) procedure of SAS [21]. The one-slope broken-line model was as follows: $Y = L - U \times (X - R)$, where L is the maximum value of parameter (asymptote), U is the slope, X is Hg concentration in diets, and R is the toxic level of Hg in diets (breakpoint × value). In addition, a regression analysis was conducted to predict daily Hg intake and Hg concentrations in the liver and breast from Hg concentrations in the feather. Significance for statistical tests was set at $P < 0.05$.

RESULTS AND DISCUSSION

Increasing concentrations of Hg in diets decreased BW (linear and quadratic, $P < 0.05$), BW gain, and FI (linear and quadratic, $P < 0.01$), but increased mortality (linear, $P < 0.01$; Table 3). The BW, BW gain, and FI for birds fed diets containing 250 mg/kg Hg were less ($P < 0.05$) than for those fed basal diets. The birds fed diets containing 500 mg/kg Hg had less ($P < 0.05$) BW, BW gain, and FI than those fed diets containing 250 mg/kg Hg. The mortality for birds fed diets containing 500 mg/kg Hg was greater ($P < 0.05$) than for those fed other diets.

The observation for the significant decrease in broiler performance by feeding diets containing greater than 250 mg/kg Hg agreed with Parkhurst and Thaxton [26] who reported that 35-d-old broiler cockerels administered drinking water containing greater than 250 mg/kg Hg from $HgCl_2$ exhibited a large reduction in BW and FI than those administered drinking water containing less than 250 mg/kg Hg. Thus, it is likely that the toxic level of Hg in diets and drinking water for broiler chickens appears to be 250 mg/kg. However, this approach using the multiple comparison of treatments with different levels of Hg in diets or drinking water has a possible limitation to determine the toxic level because the results are largely dependent of the designated treatment levels (e.g., 0, 50, 100, 250, and 500 mg/kg as used in this experiment).

To overcome this limitation, therefore, we performed the one-slope broken-line analysis to predict the toxic level of Hg in broiler diets with dietary Hg concentrations and BW gain (Figure 1). The resulting equation was $Y = 1{,}664 - 2.89 \times (X - 209)$ with an R^2 value of 0.960 ($P < 0.05$), indicating that the toxic level of dietary Hg for broiler chickens was 209 mg/kg. Thus, approximately 200 mg/kg Hg can be set to the toxic level of Hg in broiler diets, which may be more accurate than values (i.e., 250 mg/kg Hg in diets) determined based on the multiple comparison of treatment means. However, breaking points were not generated when FE and FI were used as the response variable in the current experiment. Thus, the toxic levels

Table 3. Effect of Dietary Hg Concentrations on Growth Performance of Broiler Chickens.[1]

Items[3]	Dietary Hg concentrations (mg/kg)					SEM	*P*-value[2]		
	0	50	100	250	500		T	L	Q
BW (g)	1,734[a]	1,612[b]	1,638[a,b]	1,545[b]	1,108[c]	43.3	<0.01	<0.01	0.02
BW gain (g)	1,619[a]	1,547[a,b]	1,572[a,b]	1,480[b]	1,068[c]	38.8	<0.01	<0.01	<0.01
FI (g)	2,774[a]	2,700[a]	2,691[a]	2,505[b]	1,876[c]	53.6	<0.01	<0.01	<0.01
FE (g/kg)	584	572	584	591	568	8.0	0.23	0.35	0.12
Mortality (%)	5.0[b]	9.4[b]	7.5[b]	8.8[b]	22.5[a]	3.21	<0.01	<0.01	0.21

[a-c]Means within a row with no common superscript differ significantly ($P < 0.05$) .

[1]Data are least squares means of 16 observations per treatment.

[2]T = overall effects of treatments; L = linear effects of increasing concentrations of Hg in diets; Q = quadratic effects of increasing concentrations of Hg in diets.

[3]FI = feed intake.

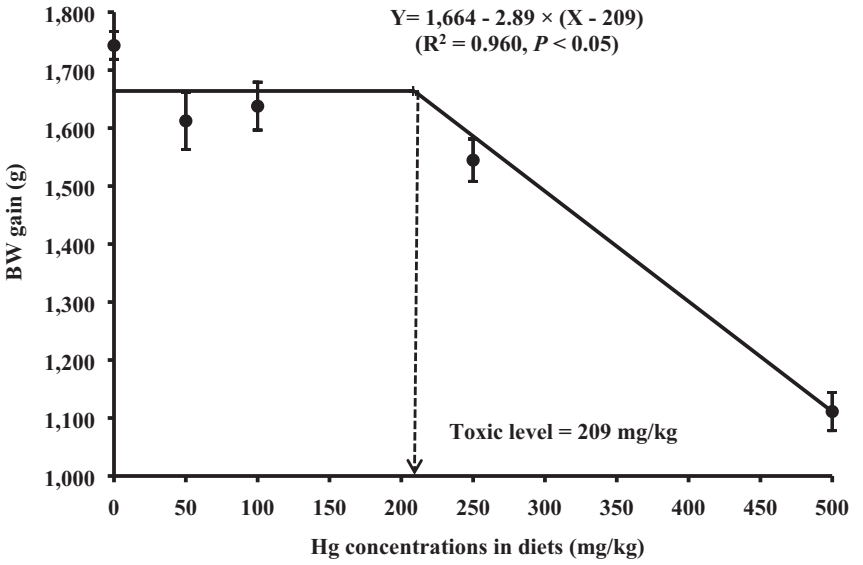

Figure 1. The one-slope broken-line analysis of BW gain at different mercury (Hg) concentrations in diets. The toxic level of Hg in broiler diets was predicted to be 209 mg/kg.

Table 4. Effect of Dietary Hg Concentrations on Hg Concentrations in the Tissue of Broiler Chickens.[1]

	Dietary Hg concentrations (mg/kg)						*P*-value[2]		
Items	0	50	100	250	500	SEM	T	L	Q
Liver (mg/kg)	0.008[b]	0.008[b]	0.010[b]	0.021[a]	0.026[a]	0.002	<0.01	<0.01	0.35
Breast (mg/kg)	0.047[d]	0.130[c,d]	0.207[c]	0.453[b]	0.893[a]	0.033	<0.01	<0.01	0.66
Feather (mg/kg)	0.115[e]	1.930[d]	2.850[c]	4.169[b]	6.085[a]	0.285	<0.01	<0.01	<0.01

[a-e]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 16 observations per treatment.

[2]T = overall effects of treatments; L = linear effects of increasing concentrations of Hg in diets; Q = quadratic effects of increasing concentrations of Hg in diets.

of dietary Hg based on the FE and FI were not calculated.

Increasing concentrations of Hg in diets increased Hg concentrations in the liver and breast (linear, $P < 0.01$), and feather (linear and quadratic, $P < 0.01$; Table 4). The Hg concentrations in the liver, breast, and feather significantly differed ($P < 0.05$) at dietary treatments of 250, 100, and 50 mg/kg Hg, respectively, when those were compared with Hg concentrations in the tissues from birds fed the basal diet. The birds fed diets containing 500 mg/kg Hg had greater ($P < 0.05$) Hg concentrations in the breast and feather than those fed diets containing 250 mg/kg Hg.

The observation for linearly increased Hg concentrations in the liver, breast, and feather with increasing Hg concentrations in diets was expected. However, a sharp increase in feather Hg concentrations for birds fed diets containing 50 mg/kg Hg resulted in a quadratic relationship, which was not case in the liver and breast. In addition, Hg concentrations in the feather were greater than those in the liver and breast among all dietary treatments. These results indicate that the feather is the major tissue for Hg accumulation and is the most responsive tissue to various Hg concentrations in diets. This observation is likely caused by the relatively low turnover rate of tissues in the feather than those in the liver and breast. Additionally, the feather can be easily and continuously collected without harming birds and in an animal-friendly way. Therefore, we suggest that the feather can be used as a prior tissue to monitor the body Hg status in broiler chickens.

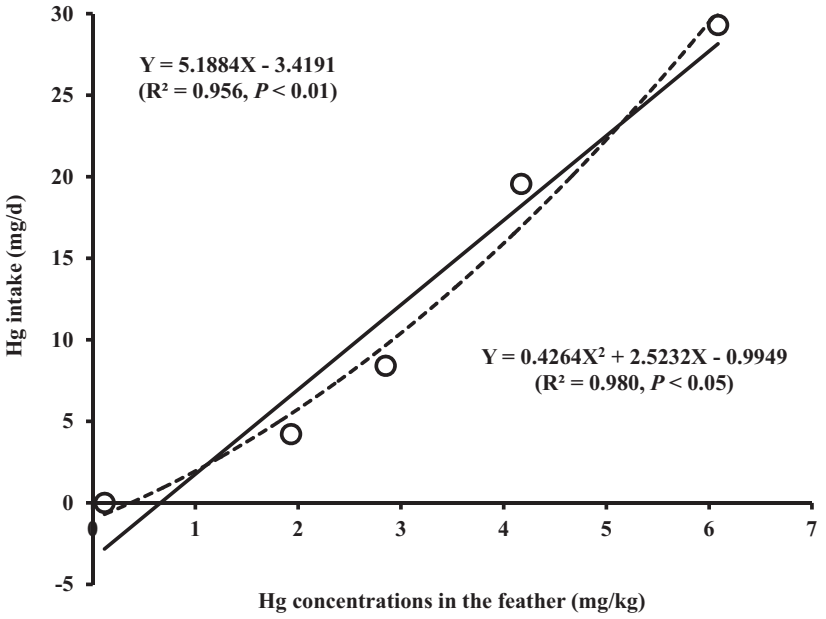

Figure 2. Linear and quadratic regression analyses for estimating Hg intake (mg/d) from Hg concentrations in the feather (mg/kg).

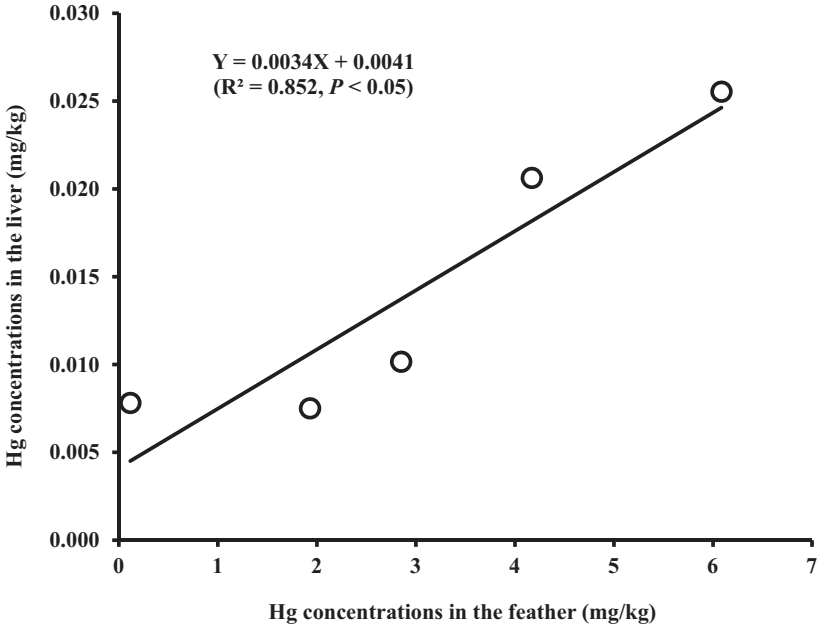

Figure 3. Linear regression analyses for estimating Hg concentrations in the liver (mg/kg) from Hg concentrations in the feather (mg/kg).

A regression analysis was performed using Hg concentrations in the feather ranging from 0.12 to 6.08 mg/kg as x variables and daily Hg intake ranging from 0 to 2.93 mg/d (Figure 2), Hg concentrations in the liver ranging from 0.008 to 0.026 mg/kg (Figure 3), and Hg concentrations in the breast ranging from 0.05 to 0.89 mg/kg (Figure 4) as y variables. Resulting

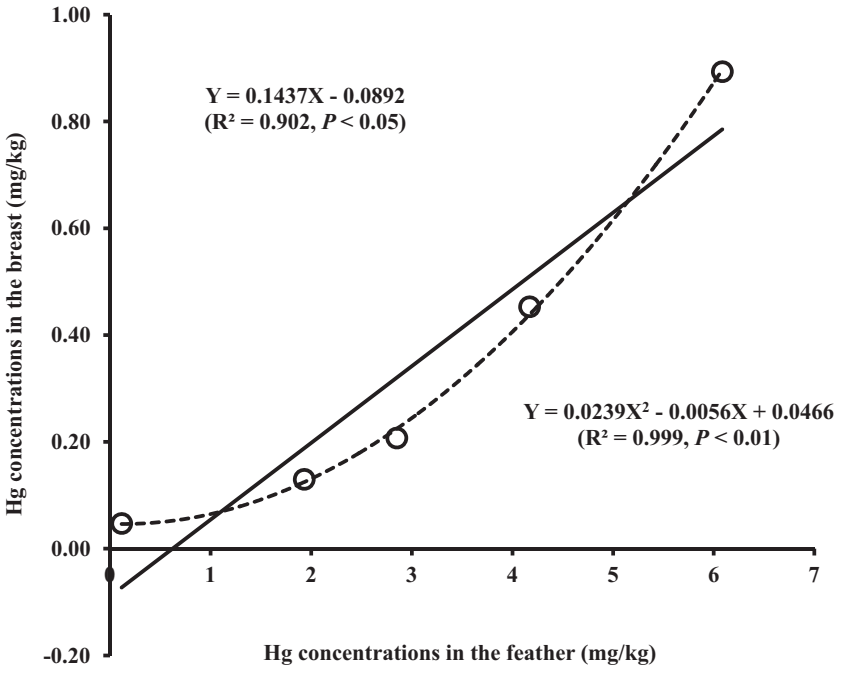

Figure 4. Linear and quadratic regression analyses for estimating Hg concentrations in the breast (mg/kg) from Hg concentrations in the feather (mg/kg).

prediction equations were $Y = 5.1884X - 3.4191$ ($R^2 = 0.956$ and $P < 0.01$) and $Y = 0.4264X^2 + 2.5232X - 0.9949$ ($R^2 = 0.980$ and $P < 0.05$) for daily Hg intake, $Y = 0.0034\ X + 0.0041$ ($R^2 = 0.852$ and $P < 0.05$) for Hg concentrations in the liver, and $Y = 0.1437X - 0.0892$ ($R^2 = 0.902$ and $P < 0.05$) and $Y = 0.0239X^2 - 0.0056X + 0.0466$ ($R^2 = 0.999$ and $P < 0.01$) for Hg concentrations in the breast.

The linear regression for predicting Hg concentrations in the liver from Hg concentrations in the feather was generated with a relatively high precision ($R^2 = 0.852$), whereas the quadratic regression was not significant. However, both linear and quadratic regressions for predicting daily Hg intake from Hg concentrations in the feather were significant with high R^2 values (0.956 and 0.980, respectively). Determining which of the 2 regressions better fits the data is difficult based on the similarities in their R^2 values and visual closeness; however, it may be more suitable to use the linear equation over the quadratic equation because of its simplicity [27, 28]. On the other hand, both linear and quadratic regressions for predicting Hg concentrations in the breast from Hg concentrations in the feather exhibited the significance with the relatively large difference in their R^2 values (0.902 and 0.999, respectively). Moreover, based on the visual fit of 2 curves and their R^2 values, it is suggested that the quadratic equation is more effective in predicting Hg concentrations in the breast from Hg concentrations in the feather than the linear equation.

CONCLUSIONS AND APPLICATIONS

1. The toxic level of Hg in diets is predicted to be near to 200 mg/kg based on BW gain in broiler chickens.
2. The feather can be a good target tissue to monitor Hg status in broiler chickens because the feather is highly responsive to different concentrations of Hg in broiler diets and is easily and continuously collected in an animal-friendly way.
3. The daily Hg intake and Hg concentrations in the liver and breast of broiler chickens

can be suitably predicted from Hg concentrations in the feather.

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