Effect of dietary melamine concentrations on growth performance, excreta characteristics, plasma measurements, and melamine residue in the tissue of male and female broiler chickens

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ABSTRACT The objectives of the current experiment were to investigate the effect of dietary melamine concentrations on growth performance, excreta characteristics, plasma measurements, and melamine residue in the tissue of male and female broiler chickens. Finally, the safe levels of melamine in broiler diets were determined based on BW gain (**BWG**) and melamine residue in the breast meat. A total of 1,008 1-d-old Ross 308 male and female broiler chickens were allotted to 1 of 7 dietary treatments within each sex in a completely randomized design. There were 6 replicates per treatment and each replicate consisted of 12 birds. Dietary melamine concentrations were set to 0; 250; 500; 750; 1,000; 5,000; or 10,000 mg/kg by adding a purified form of melamine. Diets were provided to birds on ad libitum basis for 35 d. Results indicated that no significant interaction between sex and dietary melamine concentrations was observed for all measurements. The BW, BWG, and feed intake for birds fed diets containing 10,000 mg/kg melamine were less ($P < 0.05$) than for those fed other diets. Melamine residues in the kidney and breast for birds fed diets containing 10,000 mg/kg melamine were greater ($P < 0.05$) than for birds fed other diets. The toxic level of dietary melamine based on BWG was determined by the one-slope broken-line analysis. The resulting equation was $Y = 1{,}851 - 0.0404 \times (X - 4{,}292)$, which indicated that a greater than 4,292 mg/kg melamine in diets was toxic to broiler chickens. The safe level of dietary melamine to limit melamine residue in the broiler breast was analyzed using the linear regression, which indicated that the safe level of melamine in broiler diets was 814 mg/kg. In conclusion, less than 814 mg/kg melamine in broiler diets should be maintained to satisfy human food safety regulations for melamine residue in the breast meat of broiler chickens.

Key words: broiler chicken, dietary melamine concentration, melamine residue, safe levels of melamine, sex

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INTRODUCTION

Melamine (1,3,5-triazine-2,4,6-triamine) is a 6-nitrogen (**N**)-containing organic compound. Melamine is widely incorporated into a variety of industrial materials, such as varnishes, glues, plastic packages, and food containers. Melamine is rarely metabolized by animals and is rapidly excreted via urine (Dorne et al., 2013). In many cases, therefore, very small amounts of melamine intake are not harmful to animals. The safe level of melamine concentrations in human food materials is stated as being 2.5 mg/kg by the World Health Organization (WHO, 2012) and U.S. Food & Drug Administration (FDA, 2008).

Melamine is not a normal feed ingredient, especially for nonruminant animals. However, melamine has been fraudulently added to animal diets to increase their apparent CP concentrations because melamine contains high quantities of N (i.e., 670 g/kg), and CP concentrations are determined solely by N content in the diet with the conventional Kjeldahl method. In 2007, this adulteration attracted a great public attention because many cases of nephrotoxic renal failure occurred in dogs and cats fed melamine-contaminated diets (Burn, 2007). Although it is not artificially practiced, the melamine contamination for animal diets can also occur accidently because melamine is widely used for various materials that directly contact with feed ingredients. Thus, the public pays a great attention to melamine contamination of animal diets that lead to a melamine residue in animal products for human consumption. However, previous experiments have focused primarily on mammals, and therefore, a lack of information for poultry has been available.

Previous experiments using broiler chickens have reported that birds fed diets containing up to 1,000 mg/kg melamine did not cause growth depression (Lü et al., 2009). However, those fed diets containing a greater than 10,000 mg/kg melamine had decreased BW gain

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(**BWG**) and feed efficiency (**FE**) of broiler chickens (Sirilaophaisan et al., 2010; Brand et al., 2012), suggesting that the toxic level of dietary melamine concentration is 10,000 mg/kg for broiler chickens based on their performance. As expected, melamine residue in broiler products was reported to be increased with increasing melamine intake. Lü et al. (2009) reported that increasing addition of melamine in broiler diets linearly increased the melamine residue in the breast meat, liver, and kidney. Interestingly, a longer feeding time showed less melamine residue in broiler products, indicating that broiler chickens may have a greater capacity to remove absorbed melamine with age (Lü et al., 2009). However, there is still limited information for the relationship between dietary melamine concentrations and melamine residue in broiler products. In addition, the safe limit for melamine concentrations in human foods is stated as being 2.5 mg/kg; however, the safety limit for dietary melamine concentrations that do not produce broiler products containing a greater than 2.5 mg/kg melamine have not been determined. Finally, male broiler chickens are well-known to have a greater feed intake (**FI**) than female broiler chickens (NRC, 1994). Thus, physiological change and melamine residue in response to different melamine concentrations in diets are likely different between sexes. To our knowledge, however, no experiments have compared the effects of dietary melamine concentrations on broiler chickens with different sexes.

Therefore, the objectives of the current experiment were to investigate the effect of dietary melamine concentrations on growth performance, excreta characteristics, plasma measurements, and melamine residue in the tissue of male and female broiler chickens. Finally, the safe levels of melamine in broiler diets were determined based on both growth performance and melamine residue in the breast meat.

MATERIALS AND METHODS

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

Diets, Animals and Experimental Design

The current experiment was performed using a completely randomized design with 2 × 7 factorial arrangements of sex and 7 dietary treatments. A total of 1,008 1-d-old Ross 308 male and female broiler chickens (initial BW = 46.0 ± 1.52 g) were obtained from a local hatchery (Yangji hatchery, Pyeongtaek, Republic of Korea) and were housed in 84 battery cages (76 cm × 78 cm × 45 cm = width × length × height for each cage) in an environmentally controlled room. Birds were randomly allotted to 1 of 7 dietary treatments within each sex. There were 6 replicates per treatment and each replicate consisted of 12 birds. A 2-phase feeding program with a starter diet from 0 to 21 d and a grower diet from 22 to 35 d was used. Within each phase, the basal diet was formulated to meet or exceed the NRC (1994) requirements for broiler chickens (Table 1). Dietary melamine concentrations were set to 0; 250; 500; 750; 1,000; 5,000; or 10,000 mg/kg by adding a purified form of melamine (Sigma-Aldrich Chemical Company, St. Louis, MO; ≥99.0%) at the expense of the sand in the basal diet. The experimental diets were provided to the birds on an ad libitum basis for 35 d. The diets were fed in mash form. The room temperature was maintained at 30°C during the first week and then gradually decreased to 24°C at the end of the experiment. A 24-h lighting schedule was used throughout the experiment. The BWG and FI were recorded at the end of the experiment. The FE (g/kg) was calculated by dividing BWG with FI after correction of mortality (Kim et al., 2017).

Table 1. Composition and nutrient content of experimental diets.

Items	Starter diets (0 to 3 wk)	Grower diets (4 to 5 wk)
Ingredients, %		
Corn	47.06	47.46
Soybean meal	29.30	27.34
Wheat	6.50	10.00
Corn gluten meal	6.50	4.20
Tallow	3.70	5.34
Salt	0.30	0.30
Monodicalcium phosphate	2.10	1.13
Limestone	1.40	1.68
L-threonine	0.05	0.00
DL-methionine	0.34	0.07
L-lysine HCl	0.35	0.08
Choline	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	1.00	1.00
Sand	1.00	1.00
Total	100.0	100.0
Energy and nutrient contents[3]		
AME_n, kcal/kg	3,001	3,101
Crude protein, %	22.31	20.01
Lysine, %	1.28	1.00
Methionine + Cysteine, %	1.07	0.74
Calcium, %	0.97	0.92
Nonphytate phosphorus, %	0.58	0.37

[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 $CoCO_3$), 1000 μg; I (as $Ca(IO_3)_2 \cdot H_2O$), 1.25 mg; and 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; and niacin, 60 mg.

[3]Calculated values from NRC (1994).

Data Collection and Chemical Analysis

At the conclusion of the experiment, 1 bird per replicate with a BW close to the replicate mean BW (i.e., 6 birds per treatment) was euthanized by CO_2

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Table 2. Effect of dietary melamine concentrations on growth performance in male and female broiler chickens.[1]

Items		Growth performance[2]				
		BW, g	BWG, g	FI, g	FE, g/kg	Mortality, %
Sex	Melamine, mg/kg					
Male	0	1,920	1,874	3,093	629	7.58
	250	1,958	1,912	3,132	629	4.55
	500	1,891	1,845	3,043	627	4.55
	750	1,937	1,891	3,140	626	1.52
	1,000	1,923	1,877	3,049	636	4.55
	5,000	1,922	1,876	3,067	632	6.06
	10,000	1,702	1,656	2,798	620	6.06
Female	0	1,842	1,796	2,996	622	1.52
	250	1,840	1,794	3,025	616	0.00
	500	1,839	1,793	2,938	629	1.52
	750	1,867	1,821	3,005	630	0.00
	1,000	1,834	1,788	2,977	621	0.00
	5,000	1,813	1,767	2,907	633	4.55
	10,000	1,630	1,583	2,698	611	0.00
SEM (n = 6)		36.1	35.7	48.4	6.4	2.509
Main effect						
Sex						
Male		1,893[a]	1,847[a]	3,046[a]	629	4.98[a]
Female		1,809[b]	1,763[b]	2,935[b]	623	1.08[b]
SEM (n = 42)		13.6	13.5	18.3	2.4	0.948
Melamine						
0		1,881[a]	1,835[a]	3,044[a]	625	4.55
250		1,899[a]	1,853[a]	3,078[a]	623	2.27
500		1,865[a]	1,819[a]	2,990[a]	628	3.03
750		1,902[a]	1,856[a]	3,072[a]	628	0.76
1,000		1,878[a]	1,832[a]	3,013[a]	629	2.27
5,000		1,868[a]	1,822[a]	2,987[a]	632	5.30
10,000		1,666[b]	1,620[b]	2,748[b]	615	3.03
SEM (n = 12)		25.5	25.3	34.2	4.5	1.774
Effect (*P*-value)	df					
Sex	1	<0.01	<0.01	<0.01	0.11	<0.01
Melamine	6	<0.01	<0.01	<0.01	0.19	0.63
Sex × Melamine	6	0.97	0.97	0.98	0.62	0.94

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]Data are least squares means of 6 observations per treatment.
[2]BWG, BW gain; FI, feed intake; and FE, feed efficiency (gain to feed ratio).

asphyxiation and immediately dissected. Blood samples were immediately collected from each of the selected birds via heart puncture into a 10 mL sodium heparin tube (Becton Dickinson and Co, Franklin Lakes, NJ, USA), and then centrifuged at 3000 × *g* at 4°C for 20 min to obtain the plasma. The supernatant was collected and then stored at −20°C before analysis. The concentrations of alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), gamma-glutamyl transferase (**GGT**), and total protein in the plasma samples were analyzed by using a HITACHI automatic analyzer 7020 (Hitachi Ltd., Tokyo, Japan). The kidney and breast samples were collected for analyzing melamine concentrations. The melamine concentrations were determined using Nanospace SI-2 series HPLC system (Shiseido Co., Tokyo, Japan), according to the method as described by Brand et al. (2012). Excreta samples were collected from each cage at the end of the feeding trial for analyzing excreta moisture and N. The detailed procedure was reported previously (Kim et al., 2017). Excreta moisture concentrations were measured by drying oven at 100°C for 12 h. Excreta N concentrations were determined using the Kjehldahl method (AOAC, 1995).

Statistical Analysis

All data were analyzed by ANOVA as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Each replicate was considered an experimental unit in all analyses. Outlier data were checked using the UNIVARIATE procedure of SAS (Steel et al., 1997), but no outlier was identified. The main effects in the model included sex, and dietary melamine concentrations, and their interaction. The 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. Significance for statistical tests was set at $P < 0.05$.

The one-slope broken-line (Robbins et al., 2006; Alhotan et al., 2017) was used to predict the toxic level of melamine in broiler diets based on BWG, FI, and FE using the nonlinear regression (NLIN) procedure of SAS (SAS Institute Inc., Cary, NC). The one-slope

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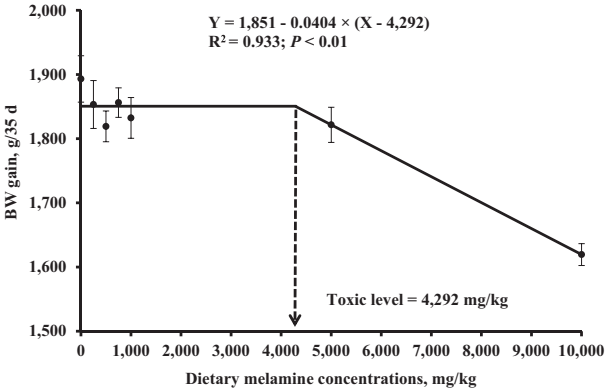

Figure 1. The one-slope broken-line analysis of BW gain (BWG) at different dietary melamine concentrations. The toxic level of melamine in broiler diets was predicted to be 4,292 mg/kg.

broken-line model was used as $Y = L - U \times (X - R)$, where L is the asymptote, U is the slope, X is melamine concentrations in diets, and R is the breakpoint x value. In addition, a linear regression analysis was performed to identify the safe limit for dietary melamine concentrations based on melamine residue in the breast and the value for food safety regulations (Chen et al., 2010).

RESULTS

In the current experiment, no significant interaction between sex and dietary melamine concentrations was observed for all measurements. Thus, the main effects of sex and dietary melamine concentrations are described herein.

Growth Performance and Excreta Characteristics

During the 5-wk feeding trial, male broiler chickens had greater ($P < 0.01$) BW, BWG, FI, and mortality than female broiler chickens (Table 2). The BW, BWG, and FI for birds fed diets containing 10,000 mg/kg melamine were less ($P < 0.05$) than for those fed other diets. However, FE and mortality were not influenced by increasing melamine concentrations in diets. The toxic level of dietary melamine based on BWG was evaluated by the one-slope broken-line model (Figure 1). The equation was $Y = 1{,}851 - 0.0404 \times (X - 4{,}292)$ with a R^2 of 0.933 ($P < 0.01$). This result indicated that the toxic level of dietary melamine based on the BWG was 4,292 mg/kg. However, when FE and FI were used as the response variable, no breaking points were generated, and thus, the toxic levels of dietary melamine based on the FE and FI were not presented.

There was no difference in excreta moisture and N concentrations between sexes (Table 3). The excreta moisture for birds fed diets containing of 10,000 mg/kg melamine was greater ($P < 0.05$) than those fed diets containing 0; 250; 500; and 1,000 mg/kg melamine. The birds fed diets containing 10,000 mg/kg melamine had greater ($P < 0.05$) excreta N than those fed diets containing 5,000 mg/kg melamine, which had greater ($P < 0.05$) excreta N than birds fed diets containing 0; 250; 500; 750; or 1,000 mg/kg melamine.

Table 3. Effect of dietary melamine concentrations on excreta moisture and nitrogen concentrations in male and female broiler chickens.[1]

		Excreta characteristics	
Items		Excreta moisture, %	Excreta nitrogen, %
Sex	Melamine, mg/kg		
Male	0	78.02	4.61
	250	79.28	4.72
	500	79.72	4.77
	750	81.22	4.91
	1,000	79.46	4.93
	5,000	81.36	5.74
	10,000	81.90	7.66
Female	0	80.60	4.92
	250	79.23	4.91
	500	79.67	5.09
	750	80.36	5.01
	1,000	80.20	4.63
	5,000	80.94	5.94
	10,000	83.44	7.62
SEM (n = 6)		1.108	0.173
Main effect			
Sex			
Male		80.14	5.33
Female		80.63	5.45
SEM (n = 42)		0.419	0.065
Melamine			
0		79.31[b]	4.77[c]
250		79.26[b]	4.82[c]
500		79.69[b]	4.93[c]
750		80.79[a,b]	4.96[c]
1,000		79.83[b]	4.78[c]
5,000		81.15[a,b]	5.84[b]
10,000		82.67[a]	7.64[a]
SEM (n = 12)		0.783	0.122
Effect (*P*-value)	df		
Sex	1	0.40	0.23
Melamine	6	0.03	<0.01
Sex × Melamine	6	0.73	0.56

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

[1]Data are least squares means of 6 observations per treatment.

Plasma Measurements

Female broiler chickens had greater ($P < 0.01$) plasma AST levels than male broiler chickens (Table 4). Plasma total protein levels for male broiler chickens were greater ($P < 0.05$) than for female broiler chickens. However, all plasma measurements were not affected by increasing melamine concentrations in diets.

Melamine Residue in the Kidney and Breast

There was no difference in the kidney and breast melamine residue between sexes (Table 5). Melamine residues in the kidney and breast for birds fed diets containing 10,000 mg/kg melamine were greater ($P < 0.05$) than for birds fed diets containing 5,000 mg/kg melamine, which had greater ($P < 0.05$) melamine

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Table 4. Effect of dietary melamine concentrations on plasma measurements in male and female broiler chickens.[1]

Items		Plasma measurements[2]			
		ALT, U/L	AST, U/L	GGT, U/L	Total protein, g/dl
Sex	Melamine, mg/kg				
Male	0	2.78	298.78	22.21	3.10
	250	3.55	316.67	25.40	3.24
	500	4.45	292.38	23.11	3.50
	750	4.28	315.98	18.61	3.56
	1,000	2.80	281.67	18.67	2.99
	5,000	2.90	298.12	20.22	3.09
	10,000	2.88	276.92	20.45	3.51
Female	0	2.33	330.45	20.65	3.02
	250	3.02	328.90	19.12	3.01
	500	2.77	380.30	19.03	3.10
	750	2.92	353.00	19.97	3.00
	1,000	2.98	316.35	17.18	2.89
	5,000	3.60	359.00	21.16	3.16
	10,000	3.23	379.22	21.12	3.04
SEM (n = 6)		0.524	33.376	1.980	0.193
Main effect					
Sex					
Male		3.38	297.22[b]	21.23	3.28[a]
Female		2.98	349.60[a]	19.75	3.03[b]
SEM (n = 42)		0.198	12.615	0.748	0.073
Melamine					
0		2.56	314.62	21.43	3.06
250		3.28	322.78	22.26	3.13
500		3.61	336.34	21.07	3.30
750		3.60	334.49	19.29	3.28
1,000		2.89	299.01	17.92	2.94
5,000		3.25	328.56	20.69	3.13
10,000		3.06	328.07	20.77	3.27
SEM (n = 12)		0.370	23.600	1.400	0.136
Effect (*P*-value)	df				
Sex	1	0.16	<0.01	0.17	0.02
Melamine	6	0.40	0.93	0.39	0.45
Sex × Melamine	6	0.21	0.82	0.41	0.64

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]Data are least squares means of 6 observations per treatment.
[2]ALT, alanine aminotransferase; AST, aspartate aminotransferase; and GGT, gamma-glutamyl transferase.

residue than those fed diets containing 0; 250; 500; 750; or 1,000 mg/kg melamine.

Linear Regression Analysis Using Melamine Concentrations in Diets and Breast

According to food safety issue (FDA, 2008; WHO, 2012), melamine concentrations of human food should be limited to less than 2.5 mg/kg. Thus, the prediction of the safe level of melamine in broiler diets was required to control and minimize melamine residue in the breast as the main food source for humans. No interaction for melamine residue in the breast between sex and dietary melamine concentrations was observed, and thus, the data from each sex were pooled to conduct a linear regression analysis. A linear relationship was found between dietary melamine concentrations and melamine concentrations in the breast. Thus, a linear regression analysis was performed using melamine concentrations in diets as x variables and melamine concentrations in the breast as y variables (Figure 2). Although there were 7 different melamine concentrations in diets, only less than 1,000 mg/kg melamine in diets were used as x variables to increase the predictability because feeding diets containing more than 5,000 mg/kg melamine increased breast melamine concentrations dramatically, which led to a large bias in regression coefficients. The linear regression revealed that melamine concentrations in the breast (Y, mg/kg) = 0.003X (melamine concentrations in diets, mg/kg) + 0.0583 ($R^2 = 0.968$; $P < 0.01$). Based on this equation, the safe level of melamine in broiler diets was estimated to be 814 mg/kg when melamine concentrations in the breast were set to be 2.5 mg/kg.

DISCUSSION

The observation for the lack of significant interaction between sex and dietary melamine concentrations in all measurements indicates that the influence of increasing melamine concentrations in broiler diets is not different between sexes. As observed in previous experiments (Buyse et al., 1996; Zuowei et al., 2011; Shim et al., 2012), male broiler chickens had greater BWG and FI than female broiler chickens in the current experiment, which is the reason why we hypothesized that different response to increasing melamine concentrations in diets

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Table 5. Effect of dietary melamine concentrations on tissue melamine residue in male and female broiler chickens.[1]

Items		Tissue melamine residue	
		Kidney, mg/kg	Breast, mg/kg
Sex	Melamine, mg/kg		
Male	0	0.15	0.10
	250	0.91	0.69
	500	1.57	0.79
	750	2.50	1.85
	1,000	3.17	2.90
	5,000	32.15	16.00
	10,000	43.63	38.23
Female	0	0.04	0.20
	250	0.36	0.81
	500	0.33	1.91
	750	0.72	3.50
	1,000	0.65	3.08
	5,000	2.74	16.77
	10,000	31.35	61.52
SEM (n = 6)		7.264	4.783
Main effect			
Sex			
Male		12.01	8.65
Female		5.17	12.54
SEM (n = 42)		2.746	1.808
Melamine			
0		0.10^{c}	0.15^{c}
250		0.64^{c}	0.75^{c}
500		0.95^{c}	1.35^{c}
750		1.61^{c}	2.68^{c}
1,000		1.91^{c}	2.99^{c}
5,000		17.44^{b}	16.39^{b}
10,000		37.49^{a}	49.87^{a}
SEM (n = 12)		5.136	3.382
Effect (P-value)	df		
Sex	1	0.08	0.13
Melamine	6	<0.01	<0.01
Sex × Melamine	6	0.37	0.16

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

[1]Data are least squares means of 6 observations per treatment.

would exist between sexes. However, the responses were not different between sexes. One possible reason may be that the difference in FI by 3.7% between sexes was too small to exert the significant physiological change in broiler chickens. Moreover, broiler chickens of both sexes used in this experiment were still in the sexually immature phase. To our knowledge, however, the current experiment is the first to report that the effects of dietary melamine concentrations are independent of sexes in broiler chickens.

In the current study, birds fed diets containing 10,000 mg/kg melamine had decreased BW, BWG, and FI compared with those fed other diets. This observation agreed with the previous experiment reporting that feeding diets containing more than 10,000 mg/kg melamine decreased BW and FI of 21-d-old broiler chickens (Brand et al., 2012). A similar observation also was observed by Sirilaophaisan et al. (2010), who reported that feeding diets containing 10,000 mg/kg melamine decreased BWG of 42-d-old broiler chickens. Additionally, a similar reduction in the growth performance of Pekin ducks also was reported (Landers et al., 2012). These results indicate that a greater than 10,000 mg/kg melamine in diets impair growth performance of broiler chickens, and therefore, this concentration in diets may be set to be a toxic level for broiler chickens based on their growth performance. However, the statistical analysis used for obtaining these results has a limitation regarding setting a toxic level of melamine in broiler diets because the analysis was based solely on multiple comparisons among treatment levels, and thus, the results depend largely on how treatment levels were designated. To overcome this limitation, we performed the one-slope broken-line analysis to predict the approximate toxic level of melamine in broiler diets (Alhotan et al., 2017). Based on this analysis, the toxic level of melamine in broiler diets was 4,292 mg/kg when BWG values were used as response variables. However, it should be noted that one-slope broken-line analysis used in this experiment assumes the linear response at the greater concentrations of melamine in diets than the breaking point (i.e., the toxic level of melamine; Robbins et al., 2006). This assumption may not be fully validated in the current experiment because there were only 2 different treatment levels with a wider difference above the toxic level of melamine in diets (i.e., 5,000 and 10,000 mg/kg). However, we assume that melamine concentrations in diets between 2 diets also lead to a linear decrease in BWG and the corresponding data stand close to the descending linear regression line. However, when FE and FI were used as the response variable, no breaking points were generated because of relatively small differences among treatment means.

It is known that absorbed melamine is rapidly excreted via urine in animals (Mast et al., 1983; Hau et al., 2009; Dorne et al., 2013). Thus, a linear increase in excreta N concentrations and accompanying water excretion was expected by increasing melamine concentrations in diets fed to broiler chickens in this experiment. However, N and water excretion were only significantly different between treatments for birds fed diets containing greater than 5,000 mg/kg melamine. The reason for this observation is unclear because the most of previous researches for body melamine metabolism have been conducted with mammals; however, it may be related to an increase in the renal dysfunction. It has been reported that high melamine intake increased diarrhea, polyuria, and proteinuria in animals in association with potential renal problems (Cianciolo et al., 2008; Dobson et al., 2008; Dalal and Goldfarb, 2011).

The plasma concentrations of ALT, AST, GGT, and total protein have been widely applied as indicators for liver functions (Lu et al., 2014; Huang et al., 2017). In the current experiment, all plasma measurements were not affected by increasing melamine concentrations in diets. This observation agreed with the previous experiment reporting that no significant difference was observed in serum AST, GGT, and total protein of broiler chickens fed diets containing 10,000 mg/kg melamine

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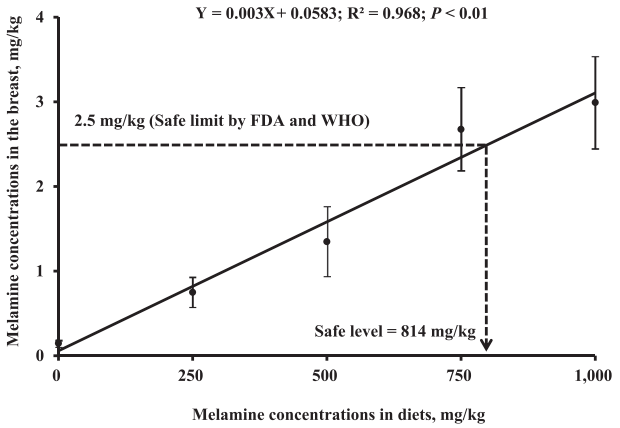

Figure 2. Linear regression analysis of melamine concentrations in the breast of broiler chickens at dietary melamine concentrations (0, 250, 500, 750, or 1,000 mg/kg). Safe limit for melamine concentrations in the breast meat is set to be 2.5 mg/kg (FDA, 2008; WHO, 2012) and the safe level of melamine in broiler diets are calculated to be 814 mg/kg.

(Brand et al., 2012). Therefore, this result indicates that birds fed diets containing from 0 to 10,000 mg/kg melamine have little negative effects on liver functions.

The melamine residue in the kidney and breast was increased for broiler chickens fed diets containing greater than 5,000 mg/kg melamine as compared to those fed other diets containing less than 1,000 mg/kg melamine. This result corroborated the findings of Brand et al. (2012), who reported significant increases in the melamine residue in the kidney and breast of broiler chickens fed diets containing greater than 5,000 mg/kg melamine. Similar increases in melamine residue in the breast by dietary melamine have also been reported in broiler chickens (Sirilaophaisan et al., 2010).

A linear regression analysis was performed using melamine concentrations in diets (0 to 1,000 mg/kg melamine) and melamine concentrations in the breast meat. The reasons for using the melamine concentrations of less than 1,000 mg/kg in diets were to improve predictability and to maintain the toxic level of broiler diets, which was predicted from BWG in the present experiment. Our results from the regression analysis suggested that every increase of 1 mg/kg melamine in diets lead to an increase of 0.003 mg/kg melamine in the breast, which indicates a transfer efficiency of dietary melamine of 0.3% for melamine in the breast meat. The safe limit for melamine concentrations in human food materials is stated as being 2.5 mg/kg by FDA (2008) and WHO (2012). Based on our equation, the melamine concentrations in diets for broiler chickens should be limited to less than 814 mg/kg to maintain melamine concentrations in the breast meat under 2.5 mg/kg. A similar regression analysis was performed by Chen et al. (2010), who analyzed the linear relationship between dietary melamine concentrations (x variables) for laying hens and melamine concentrations in eggs (y variables). There were 5 different melamine concentrations in diets from 0 to 100 mg/kg. The linear regression was Y = 0.01473X + 0.08491. Based on this equation, the safe limit for melamine concentrations in layer diets was estimated to be 164 mg/kg when melamine concentrations in eggs were set to be 2.5 mg/kg. However, there have been limited data regarding the determination of the safe limit for dietary melamine concentrations that do not produce poultry products containing a greater than 2.5 mg/kg. We suggest that the safe level of melamine or other toxic materials in animal diets should be determined based on their residue in animal products for human consumption rather than based on animal performance.

CONCLUSION

The toxic effects of dietary melamine concentrations are similar between male and female broiler chickens. The toxic level of melamine in broiler diets is 4,292 mg/kg based on the BWG of broiler chickens. However, less than 814 mg/kg melamine in broiler diets should be maintained to satisfy human food safety regulations for melamine residue in the breast meat.

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